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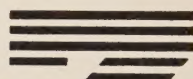
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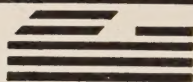
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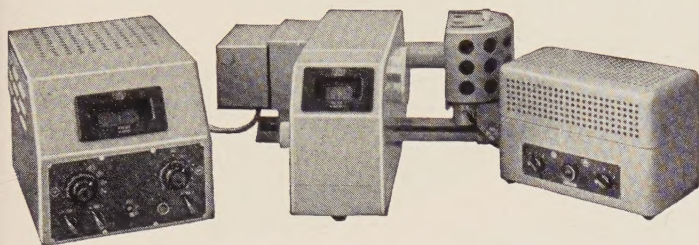
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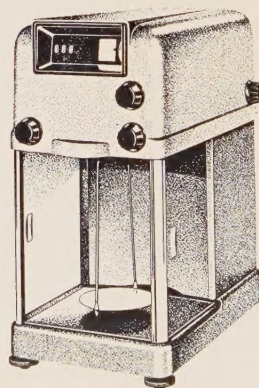
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Significance of Supraspinal Control of Reflex Actions by Impulses in Muscle Afferents

By R. M. ECCLES and A. LUNDBERG*

Work of the Sherrington school first demonstrated the importance of proprioceptive reflexes from muscle afferents in the reflex taxis of the limb¹. Subsequent investigation has been directed to discover the contributions by the various afferent systems from muscles.

Large afferents from muscle spindles (Ia fibres) are discharged by a slight stretch of the muscle. These impulses exert a monosynaptic excitatory action on motoneurons and may thereby give rise to a reflex discharge of impulses. By operation through an interneuronal relay inhibitory actions are evoked in other motoneurons by the same impulses. These excitatory and inhibitory actions have been found to be distributed to different motor nuclei according to an intricate pattern, suggesting that the Ia system subserves coordination of movements².

Large afferents from Golgi tendon organs (Ib fibres) are adequately stimulated by contraction of the muscle. Impulses in Ib fibres from extensor muscles inhibit motoneurons to the muscle of origin and to other extensor muscles, whereas flexor motoneurons receive excitation³. However, the pattern does not conform entirely with the flexion reflex. For example, Ib impulses from a flexor muscle do not evoke excitation in neurons to its own muscle.

Finally the reflex actions by the small afferents from muscle spindles ($12 - 4 \mu$), i.e. group II fibres with flowerspray ending⁴ and also of the small group III fibres ($< 4 \mu$), which probably respond adequately mainly on nociceptive stimulation, are those of the general flexion reflex⁵. The actions by impulses in

group II fibres are of particular interest. A slight stretch of the muscle will cause a discharge in these fibres, the threshold for stretch being only slightly higher than for the Ia fibres with annulo-spiral endings on muscle spindles⁴. Thus there are two systems of afferents from muscle, which are activated adequately in the same way, but with entirely different connections to motoneurons. The question thus arises: The intricate pattern of Ia actions is seemingly very suited to subserve co-ordinated movements, yet how can it assert itself in the reflex control of movement among the often very large but non specific actions by group II impulses?

The investigations of the synaptic actions of muscle afferents discussed above were carried out in animals made spinal through section of the cord. One possibility is that reflexes in spinal animals are not representative for the intact animal. Strong evidence has already been presented to show that interneurons mediating reflexes from muscle afferents are controlled from suprasegmental structures⁶.

We have therefore compared synaptic actions by the various systems of muscle afferents in decerebrate cats before and after section of the spinal cord. It has been a consistent finding that the actions by group II and III muscle afferents which are so conspicuous in the spinal animal, are almost completely absent in the decerebrate preparation. They appear immediately when the cat is made spinal by section of the cord either in the upper cervical or upper lumbar region. In the curves of Figure 1a single gastrocnemius volley conditioned a monosynaptic testing reflex that was evoked by an afferent volley in the nerve to the knee-flexors, biceps posterior and semitendinosus. With all strengths of stimulation to the gastrocnemius nerve there were no actions before the cord section, but large facilitations appeared immediately afterwards. This occurred not only for the effects evoked by stimulation of the gastrocnemius nerve at 3.0 and 15.5 times threshold for the nerve, which would activate group II and III afferents respectively, but also for the facilitatory action from stimulation at 1.6 times threshold.

* Department of Physiology, The Australian National University, Canberra (Australia).

¹ C. S. SHERRINGTON, *The integrative action of the nervous system* (Yale University Press, New Haven 1906). - R. S. CREED, D. DENNY-BROWN, J. C. ECCLES, E. G. T. LIDDELL, and C. S. SHERRINGTON, *Reflex activity of the spinal cord* (Clarendon Press, Oxford 1932).

² D. P. C. LLOYD, *J. Neurophysiol.* 9, 439 (1946). - J. C. ECCLES, R. M. ECCLES, and A. LUNDBERG, *J. Physiol.* 137, 22 (1957). - R. M. ECCLES and A. LUNDBERG, *Exper.* 13, 414 (1957).

³ Y. LAPORTE and D. P. C. LLOYD, *Amer. J. Physiol.* 169, 609 (1952). - R. GRANIT, *Receptors and sensory perception* (Yale University Press, New Haven 1955). - J. C. ECCLES, R. M. ECCLES, and A. LUNDBERG, *J. Physiol.* 138, 227 (1957).

⁴ C. C. HUNT, *J. gen. Physiol.* 38, 117 (1954).

⁵ D. P. C. LLOYD, *J. Neurophysiol.* 6, 293 (1943). - L. G. BROCK, J. C. ECCLES, and W. RALL, *Proc. roy. Soc. [B]* 138, 453 (1951). - R. M. ECCLES and A. LUNDBERG, to be published.

⁶ C. JOB, *Pflügers Arch. ges. Physiol.* 256, 406 (1952).

This stimulus was just maximal for group I and the facilitation would be caused by Ib impulses. The interpretation is that the interneurons which are involved in mediating excitatory action to motoneurons by group Ib, II and III impulses, were inhibited by impulses descending from suprasegmental structures.

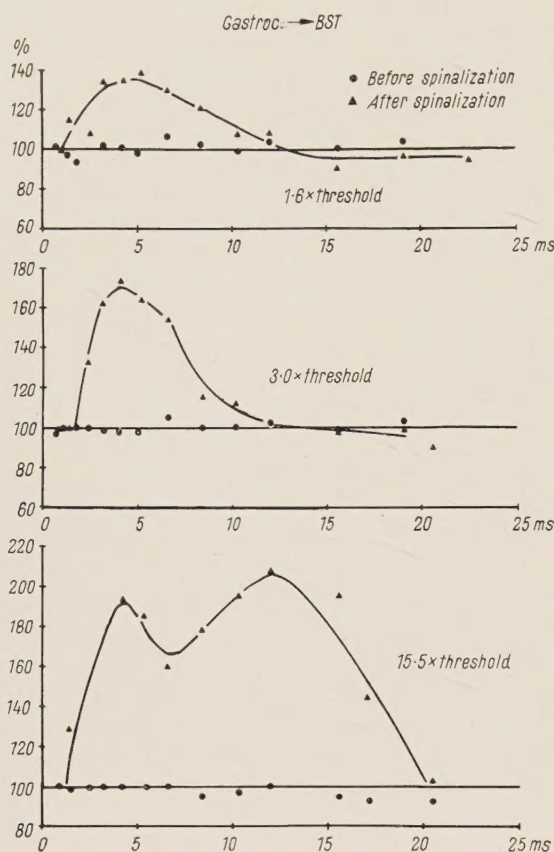


Fig. 1.—The effect of a conditioning volley in the nerve to gastrocnemius upon a monosynaptic testing reflex that was evoked by an afferent volley in the nerve to biceps posterior-semitendinosus (BST). The upper curve illustrates the action of a gastrocnemius volley which would be maximum for group I (1.6 times threshold); the lower show the effect of inclusion of group II (3.0); and group III (15.5 times threshold). The two sets of points at each strength were obtained just before (●) and just after spinalization (▲). In all curves the black line at 100% indicates the mean size of the unconditioned monosynaptic reflex.

The curves in Figure 2 show how the monosynaptic reflex from gastrocnemius was influenced by single conditioning volleys evoked at various strengths of stimulation in the nerve to flexor digitorum longus. After spinalization large inhibitory actions appeared in the two lower curves, being caused by group II and III afferent impulses respectively. Thus it is clear that interneurons mediating group II and III inhibitory actions on motoneurons of extensor muscles are also tonically inhibited from higher centres.

In the upper curve of Figure 2 stimulation at 1.55 times threshold was just maximal for group I and the inhibitory action appearing with spinalization presumably was evoked by impulses in Ib fibres. It has

been a regular finding that the Ib inhibitory actions increased with spinalization, but usually these actions were observed before spinalization. It is of particular interest that, whereas in the spinal preparation the Ib inhibitory action from flexor digitorum longus usually was larger than those elicited from gastrocnemius or

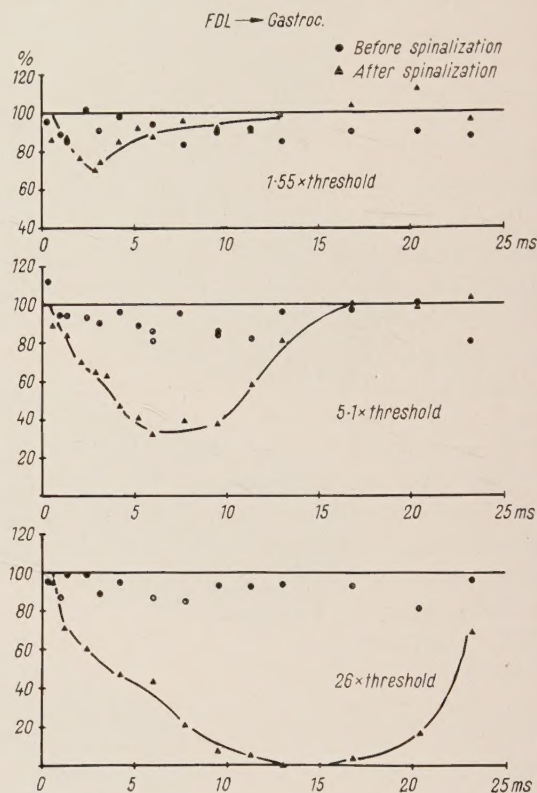


Fig. 2.—This is similar to Figure 1 but the monosynaptic testing reflex was evoked by volleys in the gastrocnemius nerve and the conditioning volleys in the nerve to flexor digitorum longus (FDL) at maximum for group I (1.55); on inclusion of group II (5.1) and group III (26 times threshold).

plantaris, the reverse occurred in the decerebrate preparation. This is demonstrated in Figure 3, where there are intracellular records from two plantaris motoneuro-

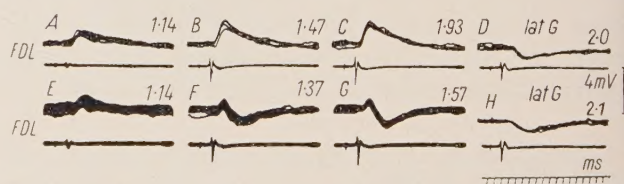


Fig. 3.—These intracellular records from plantaris motoneurons were obtained A–D before and E–H after spinalization. A–C and E–G show the effect of increasing the strength of stimulation of the flexor digitorum longus nerve. The figures indicate the strength of the volley with respect to the threshold of the nerve. D and H are the responses to an afferent volley (maximum for group I) in the lateral gastrocnemius nerve.

nes A–C before and D–G after the animal was made spinal. With increasing strength of stimulation to the flexor digitorum longus nerve there was hardly any change in the decay of the excitatory postsynaptic

potential (A-C), showing that there was little or no inhibitory action. However, after spinalization the usual large inhibitory postsynaptic potential appeared in records F and G. On the other hand the inhibitory potential caused by Ib impulses from the lateral gastrocnemius-soleus nerve appeared before as well as after section of the cord.

It has been assumed that the functions of the Ib systems in the reflex taxis of the animal would be protective, i.e. too strong a contraction of the muscle would be prevented by autogenetic inhibition⁷. However, the pattern of connections in the spinal animal suggests an additional function of the Ib action. The large inhibitory action exerted by Ib impulses from flexor digitorum longus would appear with the active plantar flexion of the toes, at the height of the extension phase of the step and could be of importance for terminating the extension phase and initiating the flexion phase of stepping.

If impulses from flexor digitorum longus normally were a contributor in the reflex regulation of stepping, these inhibitory actions would clearly require control from higher centres, for example during voluntary contraction of the flexor digitorum longus or during running. A control at the interneuronal level would certainly be most effective. Such an inhibitory control presumably exists, although less pronounced, for Ib

inhibitory actions from other muscles than flexor digitorum longus.

The functional implication of the suprasegmental control of interneurons mediating group II and III impulses may be a different one. Attention was drawn above to the co-existence in the spinal preparation of the entirely different pattern of synaptic actions caused by the two types of muscle spindle afferents, groups Ia and II respectively. In the present experiment there was no evidence that the synaptic linkage between Ia fibres and motoneurons was controlled from higher centres. On the other hand, with the interneurons mediating the group II and III actions, there exist very effective inhibitory control mechanisms from supraspinal centres. It may tentatively be suggested that in normal locomotion there is assistance by reflex actions from the Ia and Ib systems, but on account of this inhibition at the internuncial level impulses in group II and III fibres are largely without synaptic actions on motoneurons.

Zusammenfassung

An decerebrierten und spinalen Katzen wurden diverse somatische Afferenzen in ihren synaptischen Wirkungen auf Vorderhornzellen verglichen. Bei der decerebrierten Katze waren deren erregende und hemmende Wirkungen stark herabgesetzt oder nicht nachweisbar. Somit werden Zwischenneurone im Reflexbogen durch supraspinale Zentren tonisch gehemmt. Die Bedeutung dieser Kontrolle für die Reflexregulierung wird besprochen.

⁷ R. GRANIT, *Receptors and sensory perception*. Yale University Press, New Haven (1955).

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Selective Depletion of Nor-Adrenaline in the Adrenal Medulla of the Rat After Administration of Reserpine (Histochemical Research)

Recent research (CARLSSON and HILLARP¹, KRONEBERG and SCHÜMANN², MOLINATTI *et al.*³) has demonstrated that the alkaloids of '*Rauwolfia serpentina*' cause the liberation of catechol amines by the adrenal medulla of animals of different species.

In the rat, the administration of reserpine causes a definite modification of cellular chromaffinity; alongside

various groups of cells completely devoid of pheochrome substance, the remaining glandular parenchyma preserves a normal degree of chromaffinity (MOLINATTI *et al.*³). As demonstrated by HILLARP *et al.*⁴ and ERÄNKÖ⁵, adrenaline and nor-adrenaline in the rat's medulla are contained in two distinct types of cells. Therefore the particular behaviour observed in this animal has suggested to us that under the action of reserpine only one of the two catechol amines is selectively secreted.

This investigation therefore was carried out in order to examine the possible differences in behaviour of adrenaline and nor-adrenaline. The latter may be demonstrated histologically by means of HILLARP-HÖKFELT's reaction⁶, which consists in the formation of a pigment insoluble in

¹ A. CARLSSON and N. A. HILLARP, Kungl. Physiogr. Sällsk. Lund. Förh. 8, 26 (1956).

² G. KRONEBERG and H. J. SCHÜMANN, Arzneimittelforschung 4, 279 (1957).

³ G. M. MOLINATTI, F. CAMANNI, and O. LOSANA, Arch. int. Pharmacodyn. (in press).

⁴ N. A. HILLARP and B. HÖKFELT, Endocrinology 55, 255 (1954).

⁵ O. ERÄNKÖ, Endocrinology 57, 363 (1955).

⁶ N. A. HILLARP and B. HÖKFELT, J. Histochem. Cytochem. 3, 1 (1955).

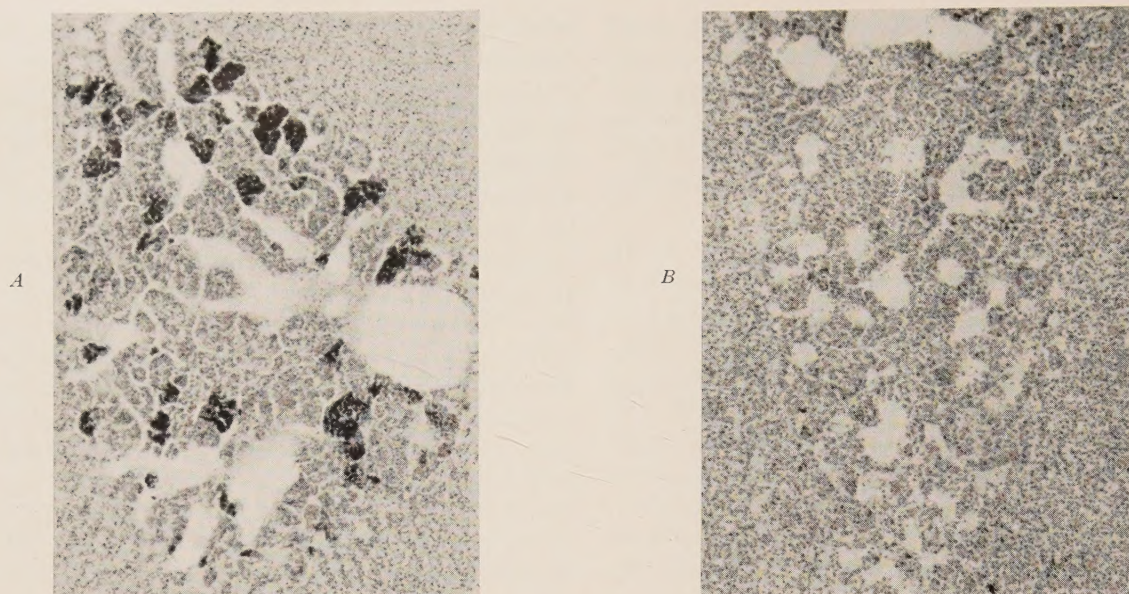


Fig. 1.— Adrenal medulla of rat before and after treatment with reserpine. Hillarp-Hökfelt's reaction. In *A*, the cellular groups appear containing a dark yellow pigment which disappears entirely after administration of reserpine (*B*). 65 $\times$.

water due to the oxidation of nor-adrenaline with potassium iodate; whereas oxidation with chromic salts enables the two catecholamines to be demonstrated simultaneously.

Methods.—20 albino rats, Wistar stock, male sex, average weight 200 g were used. The animals were divided into two groups and treated with reserpine, in doses of 0.5 mg and 1 mg/kg body weight respectively. The injections were made endoperitoneally and were repeated for 3 consecutive days to the animals of the first group and for 5 days to those of the second group. A control group was given injections of physiological solution in the same way.

At the end of the treatment all the animals were killed by decapitation. The adrenals glands were immediately removed and cut into thin sections; some were immersed for 48 h in a 10% solution of potassium iodate (HILLARP's specific reactive for nor-adrenaline) and then, after fixation in formalin, cut with the freezing microtome; others were immersed for 3 days in a solution of chromic salts

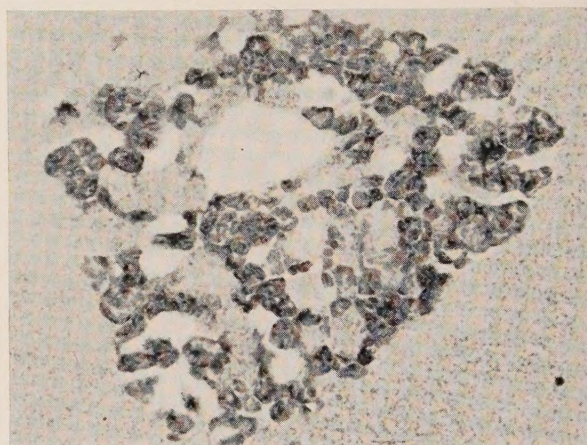


Fig. 2.— Adrenal medulla of rat. Chromaffin reaction. After administration of reserpine cellular islets completely free from pheochromic granules are encountered. 65 $\times$.

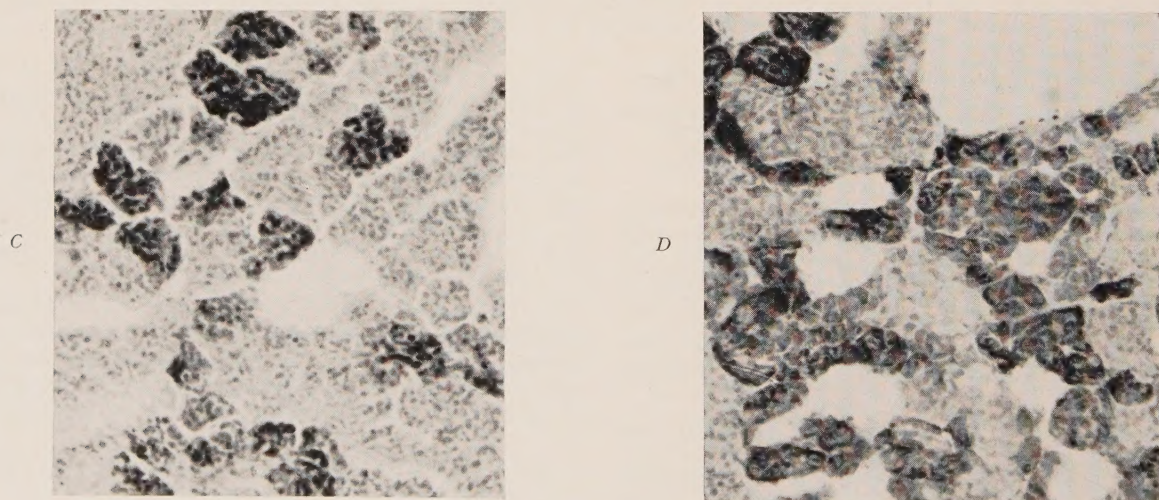


Fig. 3.— Detail of Figure 1A (*C*) and Figure 2 (*D*). 130 $\times$.

(10 parts of 5% potassium bichromate + 1 part of 5% potassium chromate) and afterwards enclosed in paraffin.

Results.—In the control group the sections of adrenal medulla treated with chromic salts showed a normal degree of chromaffinity in all the cells, while in those treated with HILLARP's reagent cellular groups were evident containing a dark yellow pigment and irregularly distributed in the remaining parenchyma, which was completely devoid of pigment (Fig. 1A, 3C). In each instance the adrenals of the treated rats showed a histochemical picture strikingly different from that of the normal gland. When doses of 0.5 mg/kg were used, with the chromaffin reaction cellular islets could be observed in the adrenal medulla completely free from pheochrome granules (Fig. 2, 3D). These cellular groups were irregularly distributed; the number of their cellular elements was quite variable. However most of the parenchyma preserved a normal degree of chromaffinity. A sudden passage, without intermediary stages, was always observed between the groups of non-chromaffin cells and the adjacent cellular columns, which were intensely pheochrome. With the HILLARP's reaction potassium iodate, however, it was never possible to show, in any cell, the formation of the characteristic dark pigment (Fig. 1B). On the contrary, the administration of reserpine in stronger doses (1 mg/kg for 4–5 consecutive days) caused the almost complete disappearance of the chromaffinity of every glandular element; the cellular cytoplasm appeared more or less vacuolised, relatively basophil, and completely devoid of pheochrome granules. With intermediate doses increasing progressively from 0.5 mg to 1 mg/kg, a progressive reduction was noted in the number of cells positive to chromaffin reaction; in all these stages HILLARP's reaction was consistently negative.

Discussion.—In agreement with what has been demonstrated by other authors by chemical methods (CARLSSON and HILLARP¹, KRONEBERG and SCHÜMANN²) and by our previous research³, it may be considered, from examination of the histochemical findings described, that the administration of reserpine causes a more or less intense diminution of the catechol amine content in the adrenal medulla. The size of this discharge seems proportionate to the dose of alkaloid administered, to the point of reaching with the highest dosage, the complete depletion of the catecholic content of the gland.

The finding of total disappearance of dark yellow pigment after oxidation with potassium iodate in the cellular tissue of the rats treated with reserpine in doses of 0.5 mg/kg denotes a complete depletion of nor-adrenaline; and since, under these conditions, the chromaffin reaction still demonstrates a high number of positive cells, it may be deduced that the adrenaline content has not, on the contrary, undergone any notable modifications. On the other hand, by comparing of the sections treated with chromic salts with those subjected to oxidation with potassium iodate, it can be observed that the number and the topographical distribution of cellular elements devoid of pheochrome material correspond fairly well the number and the distribution of the cellular elements which, in the normal gland, seem to be rich in pigment with HILLARP's reaction.

Therefore it can be stated that, in the above-considered doses and within the limitations imposed by the histochemical reactions adopted, reserpine causes an almost selective discharge of nor-adrenaline; with stronger doses, besides the liberation of nor-adrenaline, there seems to be also a progressive diminution of adrenaline up to the complete disappearance of all the catecholic content.

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Institute of Medical Pathology of the University of Turin (Italy), March 2, 1958.

Riassunto

Con tecniche istochimiche è stata indagata l'azione della reserpina sul contenuto catecolico della midollare surrenale del ratto. Viene documentata una scarica pressochè selettiva di nor-adrenalina.

**Histochemische Untersuchungen
über den Einfluss von Iproniazid (Marsilid) auf
die durch Reserpin erzeugte Freisetzung von
Adrenalin und Noradrenalin aus dem
Nebennierenmark**

Chemische und histochemische Untersuchungen ergaben, dass Iproniazid durch Hemmung der Monoaminoxidase (MO) *in vivo* nicht nur den Abbau von «freiem» 5-Hydroxytryptamin (5HT, Serotonin) verhindert¹, sondern möglicherweise auch die nach Injektion von Reserpin feststellbare Freisetzung des gespeicherten 5HT aus den Körperdepots hemmt². Grosse Dosen Reserpin setzen auch den Katecholamingehalt verschiedener Gewebe³ und des Blutplasmas⁴ herab, eine Wirkung, die im Gehirn durch Iproniazid-Vorbehandlung signifikant abgeschwächt wird⁵. In der vorliegenden Arbeit soll mit histochemischen Methoden geprüft werden, ob eine solche Abschwächung der Reserpinwirkung an den Katecholaminen des Nebennierenmarkes der Ratte sichtbar gemacht werden kann.

Versuchsanordnung. Gruppen von 8 bis 12 männlichen oder weiblichen, 80 bis 120 g schweren Wistar-Ratten eigener Zucht wurden wie folgt behandelt (Tabelle I):

Iproniazid wurde als Phosphat (Marsilid*), Isoniazid als Base (Rimifon*) in äquimolarer Dosierung verwendet. 24 h nach der Reserpininjektion wurden die Ratten getötet (Kontrollgruppen VIII und IX gleichzeitig mit Gruppen VI und VII), die Nebennieren herauspräpariert, einmal zerschnitten und lebensfrisch fixiert. Der *histochemische Nachweis der Katecholamine* erfolgte nach folgenden Methoden: Gruppen I bis V: Fixation in Müllerscher Lösung (Kaliumbichromat 2,5 – Natriumbisulfat 1,0 – Aqua dest. *ad* 100,0) während 3 Tagen, Paraffineinbettung und Gegenfärbung mit Haemalaun.

Gruppen VI bis IX (nach der Vorschrift von HILLARP und HÖKFELT⁶): Eine Nebenniere pro Tier, während 24 h

* Fabrikmarke.

¹ E. A. ZELLER, J. BARSKY, J. R. FOUTS, W. F. KIRCHHEIMER und L. S. VAN ORDEN, *Exper.* 8, 349 (1952). – E. A. ZELLER, J. BARSKY und E. R. BERMAN, *J. biol. Chem.* 214, 267 (1955). – A. SJOERDSMA, T. E. SMITH, T. D. STEVENSON und S. UDENFRIEND, *Proc. Soc. exp. Biol. Med.* 89, 36 (1955).

² A. PLETSCHER, *Helv. physiol. Acta* 14, C 76 (1956); *Exper.* 12, 479 (1956). – G. ZBINDEN, A. PLETSCHER und A. STUDER, *Klin. Wschr.* 35, 565 (1957).

³ M. HOLZBAUER und M. VOGT, *J. Neurochem.* 1, 8 (1956). – A. BERTLER, A. CARLSSON und E. ROSENGREN, *Naturwissenschaften* 43, 521 (1956). – Y. TAKETOMO, P. A. SHORE, E. G. TOMICH, R. KUNTZMAN und B. B. BRODIE, *J. Pharmacol. exp. Ther.* 119, 188 (1957). – B. B. BRODIE, J. S. OLIN, R. G. KUNTZMAN und P. A. SHORE, *Science* 125, 1293 (1957). – A. PLETSCHER, *Schweiz. med. Wschr.* 87, 1532 (1957).

⁴ M. BURGER, *Helv. physiol. Acta* 14, C13 (1956); *Arch. exp. Path. Pharm.* 230, 489 (1957).

⁵ A. PLETSCHER, *Schweiz. med. Wschr.* 87, 1532 (1957).

⁶ N. A. HILLARP und B. HÖKFELT, *Acta physiol. scand.* 30, 55 (1954); *J. Histochem. Cytochem.* 3, 1 (1955).

Tabelle I

Gruppe	Anzahl (Ratten)	Geschlecht	Behandlung		
			1. Tag, 17 h	2. Tag, 8 h	2. Tag, 14 h
I	8	W	100 mg/kg Iproniazid intraperitoneal	—	10 mg/kg Reserpin
II	8	W	—	—	10 mg/kg Reserpin
III	10	W	100 mg/kg Iproniazid intraperitoneal	—	10 mg/kg Reserpin
IV	10	W	50 mg/kg Isoniazid intraperitoneal	—	10 mg/kg Reserpin
V	10	W	—	—	10 mg/kg Reserpin
VI	12	M	100 mg/kg Iproniazid intraperitoneal	10 mg/kg Reserpin intraperitoneal	—
VII	12	M	—	id.	—
VIII	12	M	100 mg/kg Iproniazid intraperitoneal	—	—
IX	6	M	—	—	—

eingelegt in 2,5prozentiger Lösung von Kaliumjodat in Acetatpuffer bei pH 6, 48 h in 5% Formol nachfixiert, Gefrierschnitt. Selektive Darstellung der Noradrenalin-haltigen Zellen. Die andere Nebenniere 24 h eingelegt in ungepufferte Kaliumbichromatlösung (2,5prozentig mit Zusatz von Kaliumbichromat bis zu einem pH zwischen 5 und 6), 48 h Nachfixation in 5% Formol, Gefrierschnitt. Darstellung sowohl der Adrenalin- wie der Noradrenalin-haltigen Zellen.

Bei der *mikroskopischen Beurteilung* der histochemischen Katecholamin-Reaktionen (Tab. II) wurde die Intensität der Braunfärbung wie folgt bewertet:

- +++ Intensive Braunfärbung aller positiv reagierenden Zellen
- ++ Mässige Braunfärbung der meisten positiv reagierenden Zellen
- + Starke Abschwächung der histochemischen Reaktionen
- (+) Nur noch wenige Zellen mit schwach positiven Reaktionen
- 0 Keine positive Reaktion oder nur ganz vereinzelte Zellen schwach angefärbt.

Ergebnisse. Mit Ausnahme der 7 von 30 mit Iproniazid vorbehandelten Ratten, die vorzeitig starben, konnten alle Tiere histopathologisch untersucht werden. Die Beurteilung der histochemischen Katecholamin-Reaktionen im Nebennierenmark ergibt sich aus Tabelle II. Die Chromaffinität (Reaktion von Adrenalin und Noradrenalin) ist bei den nur mit Reserpin behandelten Ratten (Gruppen II, V, VII) deutlich abgeschwächt. Sehr oft sind nur noch ganz vereinzelte Nebennierenmarkzellen schwach gelb gefärbt (Abb. 1). Die histochemische Noradrenalin-Reaktion mit Kaliumjodat ist praktisch nega-

tiv. Bei den mit Iproniazid vorbehandelten Ratten (Gruppen I, III, VI) dagegen sind die histochemischen Katecholamin-Reaktionen entweder ebenso stark positiv wie bei den Kontrollen oder, in einzelnen Fällen, nur in geringem Grade abgeschwächt (Abb. 2). Die Vorbehandlung mit äquimolaren Isoniaziddosen (Gruppe IV) hat im Vergleich zu den entsprechenden Kontrollen (Gruppe V) ebenfalls eine gewisse Abschwächung der Reserpinwirkung auf das Nebennierenmark zur Folge. Der Effekt ist jedoch viel weniger deutlich als mit Iproniazid. – Die Tiere der Gruppe VIII wurden nur mit Iproniazid behandelt. Eine Veränderung der Chromaffinität und der Kaliumjodat-Reaktion des Nebennierenmarkes lässt sich bei ihnen erwartungsgemäss nicht feststellen. Degenerative Veränderungen des Nebennierenmarkes sind bei keinem Tier nachweisbar. Die durch Reserpin erzeugte Ausschüttung der Katecholamine erfolgt somit ohne histologisch erkennbare Schädigung der Zellen.

Diskussion. Obwohl Adrenalin und Noradrenalin wahrscheinlich nicht nur durch die MO abgebaut werden⁷, bewirkt die Hemmung dieses Fermentes deutliche Veränderungen im Stoffwechsel der Katecholamine, so namentlich eine Zunahme des Adrenalin- und Noradrenaliningehaltes verschiedener Organe⁸. Histochemisch lassen sich die Katecholamine im Nebennierenmark durch die chromaffine Reaktion und die Oxydation mit Kaliumjodat

⁷ E. A. ZELLER, J. BARSKY und E. R. BERMAN, J. biol. Chem. 214, 267 (1955). – E. C. GRIESEMER, J. BARSKY, C. A. DRAGSTEDT, J. A. WELLS und E. A. ZELLER, Proc. Soc. exp. Biol. Med. 84, 699 (1953). – E. A. ZELLER, J. BARSKY, E. R. BERMAN und J. R. FOUTS, J. Allergy 25, 69 (1954). – S. J. CORNE und J. D. P. GRAHAM, J. Physiol. 135, 339 (1957).

⁸ A. PLETSCHER, Schweiz. med. Wschr. 87, 1532 (1957); Exper. 14, 73 (1958).

Tabelle II

Guppe	Tierzahl (Ratten)	Vorbehandlung		Reserpin 10 mg/kg intraperitoneal	Spontan gestorben	Intensität der chromaffinen Reaktion (Anzahl Ratten) +++ ++ + (+) 0	Intensität der Kaliumjodat- Reaktion (Anzahl Ratten) ++ + (+) 0
		Iproniazid 100 mg/kg intraperitoneal	Isoniazid 50 mg/kg intraperitoneal				
I	8	+	—	+	0	2 5 1 0 0	— — — —
II	8	—	—	+	0	0 1 3 3 1	— — — —
III	10	+	—	+	1	7 2 0 0 0	— — — —
IV	10	—	+	+	0	1 3 1 5 0	— — — —
V	10	—	—	+	0	0 1 3 3 3	— — — —
VI	12	+	—	+	6	3 3 0 0 0	1 2 3 0
VII	12	—	—	+	0	0 4 3 4 1	0 0 3 9
VIII	12	+	—	—	0	12 0 0 0 0	10 2 0 0
IX	6	—	—	—	0	6 0 0 0 0	4 1 1 0

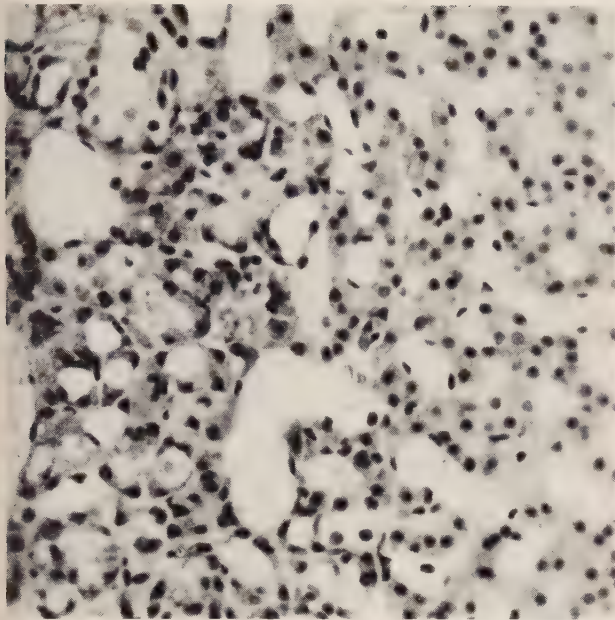


Abb. 1. Nebenniere (Rinde-Mark-Grenze), Ratte. Fixation. K.bichromat-Formol. Gegenfärbung mit Haemalaun; Vergr. 340mal. Status 24 h nach 10 mg/kg Reserpin intraperitoneal. Weitgehender Schwund der Chromaffinität in Nebennierenmark (links im Bild).

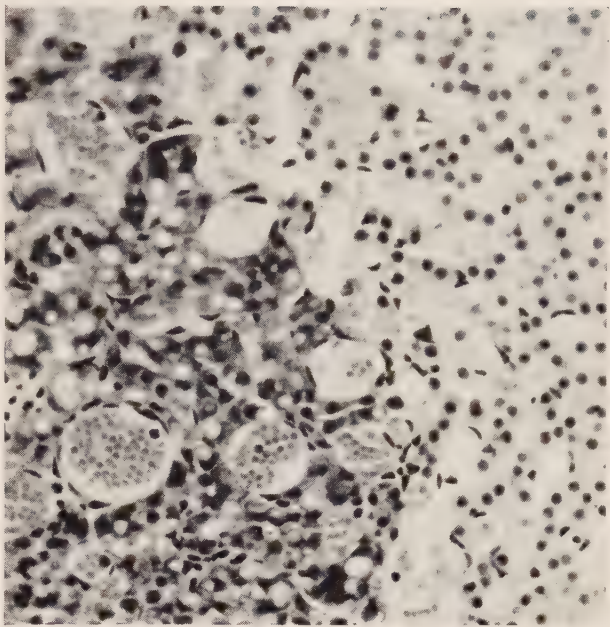


Abb. 2. Nebenniere (Rinde-Mark-Grenze), Ratte. Fixation K.bichromat-Formol. Gegenfärbung mit Haemalaun; Vergr. 340mal. Behandlung mit 100 mg/kg Iproniazid intraperitoneal. 21 h später mit 10 mg/kg Reserpin intraperitoneal. Status 24 h nach Reserpininjektion. Chromaffinität des Nebennierenmarks erhalten (links im Bild).

leicht darstellen⁹. Damit wird es auch möglich, den bei Belastung mit grossen Reserpindosen auftretenden Schwund der Katecholamine sichtbar zu machen. Bei Hemmung der MO durch Iproniazid wird die Wirkung des Reserpins auf den Adrenalin- und Noradrenalinstoffwechsel deutlich vermindert: Der Gehalt dieser beiden Stoffe sinkt im Gehirn weniger stark ab⁵, und beide histochemischen Katecholamin-Reaktionen des Nebennierenmarkes sind nicht oder nur wenig abgeschwächt. Aus diesen Befunden darf geschlossen werden, dass die MO bei der Freisetzung der Katecholamine aus den Speicherzellen eine gewisse Rolle spielt. Für diese Annahme spricht auch die Beobachtung, dass nach Vorbehandlung mit Isoniazid, welches die MO in viel geringerem Masse hemmt¹⁰, die Reserpin-bedingte Ausschüttung der Katecholamine aus dem Nebennierenmark fast ebenso ausgeprägt ist wie bei nicht vorbehandelten Ratten. Die Verhältnisse sind somit sehr ähnlich wie beim 5HT: Auch dieses Monoamin wird, wie chemische Bestimmungen¹¹ und histochemische Untersuchungen der enterochromaffinen Zellen¹² ergaben, durch Reserpin freigesetzt und beim Iproniazid-vorbehandelten Tier trotz Injektion subletaler Reserpindosen teilweise in den Speicherzellen festgehalten¹³.

Kürzlich wurde festgestellt, dass Iproniazid in vielen Fällen von Angina pectoris eine günstige therapeutische

Wirkung ausübt¹⁴. Es wurde angenommen, dass diese Wirksamkeit mit den Veränderungen des Katecholamin-Stoffwechsels in Zusammenhang steht¹⁵. Adrenalin und Noradrenalin sollen jedoch ungünstig auf den Stoffwechsel des Herzmuskels wirken¹⁶, so dass die Vermehrung dieser zwei Stoffe im Myocard die therapeutische Wirksamkeit des Iproniazid bei Coronarinsuffizienz nicht erklärt. Die vorliegenden Ergebnisse könnten nun, wie dies von PLETSCHER¹⁵ vermutet wurde, dahin interpretiert werden, dass die Hemmung der MO durch Iproniazid zwar eine Ansammlung der Katecholamine in den Organen bewirkt, jedoch die Freisetzung dieser für den Myocardstoffwechsel ungünstig wirkenden Stoffe hemmt und die Bindung an strukturelle Elemente in den Zellen verstärkt.

G. ZBINDEN und A. STUDER

Abteilung für experimentelle Medizin der F. Hoffmann-La Roche & Co. AG., Basel, 12. März 1958.

Summary

A single injection of 10 mg/kg reserpine causes marked decrease of catecholamine in the medulla of the adrenal gland of rats. Histochemically, this can be shown by the disappearance of chromaffinity and of the potassium iodate reaction. After pretreatment with 100 mg/kg of iproniazid, reserpine injection induces little or no decrease in the histochemical reactions of adrenalin and noradrenalin. In animals pretreated with equimolar doses of isoniazid, however, histochemical catecholamine reactions are well preserved in all cells of the adrenal medulla. These results suggest that monoamine oxidase plays a part in the reserpine-induced release of catecholamine.

¹⁴ T. CESARMAN, Arch. Inst. Cardiol. M  x. 27, 563 (1957); Circulation, im Druck. – P. COSSIO, Pren. m  d. Argent. 44, 2679 (1957). – W. SCHWEIZER, Vortrag Schweiz. Cardiologische Gesellschaft, 17. November 1957, Gen  ; Cardiologia 33, Nr. 1 (1958).

¹⁵ A. PLETSCHER, Exper. 14, 73 (1958).

¹⁶ W. RAAB, Adv. Cardiol. vol. 1 (S. Karger, Basel/New York 1956), p. 65.

⁹ N. A. HILLARP und B. H  KFELT, Acta physiol. scand. 30, 55 (1954); J. Histochem. Cytochem. 3, 1 (1955). – O. ER  NK  , J. Histochem. Cytochem. 4, 11 (1956); Nature 168, 250 (1951); 175, 88 (1955).

¹⁰ E. A. ZELLER, J. BARSKY, J. R. FOUTS, W. F. KIRCHHEIMER und L. S. VAN ORDEN, Exper. 8, 349 (1952). – E. A. ZELLER, J. BARSKY, E. R. BERMAN, und J. R. FOUTS, J. Pharm. exp. Ther. 106, 427 (1952). – E. A. Zeller und J. BARSKY, Proc. Soc. exp. Biol. Med. 81, 459 (1952).

¹¹ A. PLETSCHER, P. A. SHORE und B. B. BRODIE, Science 122, 374 (1955); J. Pharm. exp. Ther. 116, 84 (1955).

¹² E. P. BENDITT und R. L. WONG, Amer. J. Path. 32, 638 (1956). – G. ZBINDEN, A. PLETSCHER und A. STUDER, Schweiz. med. Wschr. 87, 629 (1957).

¹³ A. PLETSCHER, Helv. physiol. Acta 14, C 76 (1956); Exper. 12, 479 (1956). – G. ZBINDEN, A. PLETSCHER und A. STUDER, Klin. Wschr. 35, 565 (1957).

Secretion of Gastric Parietal Cells

The exact nature of the secretion of the gastric parietal cells is still debated¹. While applying a new histochemical method it was found that the parietal cells secrete large amounts of a weak polyacid². The present report deals with further investigation on this secretion following histamin stimulation.

Materials and Methods. Four mongrel dogs were fasted for 24 h and given sodium pentobarbital in doses sufficient to keep them sleeping during most of this period. Repeated doses of 5 mg diethylaminoethyl-benzilate-methobromide³, were given intramuscularly during 6 h preceding operation. This premedication was maintained in order to produce prolonged inhibition of gastric secretion⁴. At operation the ligated stomach was incised and the pH of the gastric contents determined by indicator paper; biopsy specimens were then taken. Subsequently 0.7 to 1.0 mg of histamin hydrochloride was introduced into the peritoneal cavity, and the testing for acidity and taking of biopsies at half hourly intervals were carried out 2–3 times thereafter.

The biopsy specimens were fixed in Carnoy's fluid, embedded in paraffin and sectioned at 6 microns. They were stained with haematoxylin and eosin and by the Bi-Col method², which involves the use of 2 colloidal metal solutions. The colloidal iron is used in order to stain and block the strongly acidic groups, followed by staining with colloidal gold, which can now only react with the remaining weakly acidic compounds. Some series of sections were stained by the P.A.S. method, by the Ninhydrin-Schiff and the Million methods.

Results. The reaction of the gastric contents before histamin administration was neutral. 30–60 min later the reaction became acid (pH 1–4). Concomitant with the change in secretion, the Bi-Col method revealed changes in the appearance of the glands, which were similar in all the dogs but most clear in the dog from which the microphotographs of Figure 1 were taken.

In all the biopsy specimens blue-staining material (indicating strongly acidic groups, i.e. mucus) was found in the surface epithelium, in the foveolae and in the necks of the glands. In the first specimen (Fig. 1A, reaction neutral) dark brown material, indicating a high concentration of a weak acid, was found in the uppermost part of the glandular lumen and in a few parietal cells in this region. In the second specimen (Fig. 1B) the appearance was essentially similar. In both these specimens all the microscopical fields revealed the same picture. In the third biopsy taken 1 h after histamin stimulation (Fig. 1C, pH around 2) dark brown material was found in the lumina of the middle and upper parts of some glands. It was also found in some parietal cells, lying apparently within the cytoplasm or in the intracellular canaliculi. In the 4th specimen (Fig. 1D, pH 1–2), the dark brown material was found along the entire length of the lumina as well as in most parietal cells. It may be noted that in the last 2 specimens areas were found which did not differ markedly from those observed in the first 2 specimens.

In P.A.S.-stained preparations there was diffuse staining of the parietal cells and of the secretion within the lumina. No appreciable staining of the described material

was observed in Ninhydrin-Schiff and Millon stained preparations.

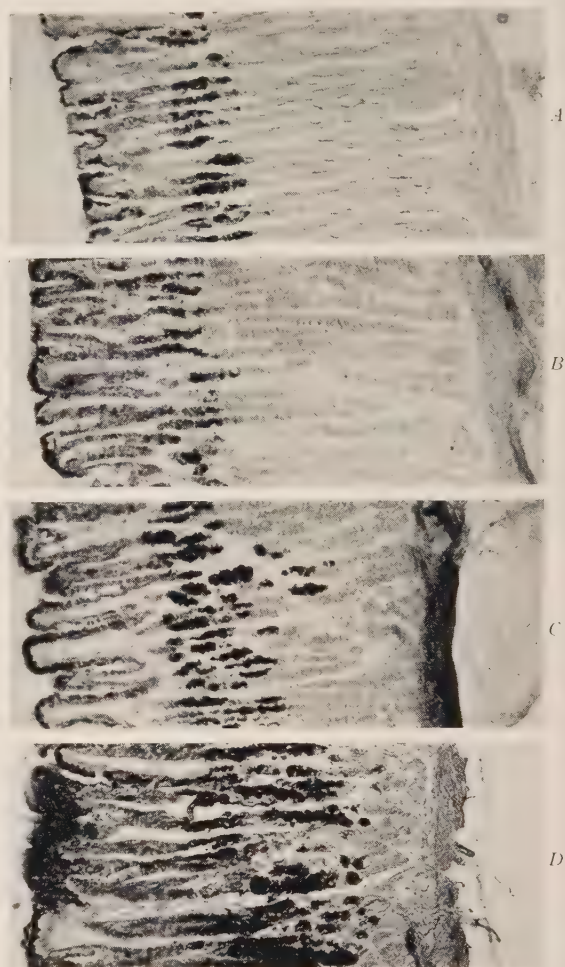


Fig. 1. Consecutive biopsy specimens of gastric mucosa of a dog. Bi-Col stain, $\times 120$. Mouths of the gastric glands are to the left; here the dark staining material is mucus which is colored blue by the Bi-Col method. Dark staining material within the glands is mucoid which is stained dark brown by the same method.

Fig. 1A. First biopsy taken after long anaesthesia and administration of a ganglion blocking agent. Mucosa apparently at rest (reaction of gastric contents neutral), with dark brown granules in the uppermost parts of the glands.

Fig. 1B. Second biopsy, taken $1\frac{1}{2}$ h after administration of histamine. Appearance of mucosa similar to A. (Reaction of gastric contents neutral.)

Fig. 1C. Third biopsy, taken 1 h after histamine administration. Dark brown material is present also in the middle portion of the glands, both in the lumina and in the parietal cells. (Reaction of the gastric contents acid; pH about 2.)

Fig. 1D. Fourth biopsy, $1\frac{1}{2}$ h after histamine stimulation. Dark brown material is present throughout glands in lumina and parietal cells. (Reaction of gastric contents acid; pH 1–2.)

Discussion. These findings indicate that the gastric parietal cells of the dog stimulated by histamin contain and secrete a substance which is demonstrable by the Bi-Col method. In apparently resting glands the substance is present only in the upper parts of the glands, where it borders on the mucus plug.

The material is obviously insoluble in the organic solvents used for fixation and embedding and apparently does not diffuse to a great extent during fixation. Previous

¹ E. J. CONWAY, *The Biochemistry of Gastric Acid Secretion* (C. C. Thomas, Springfield, Ill., 1953).

² M. WOLMAN, *Bull. Res. Council Israel* 6E, 27 (1956).

³ 'Paragone' Teva, Jerusalem.

⁴ D. BIRNBAUM, *Gastroenterologia* 82, 225 (1954).

(unpublished) experiments have indicated that it is moderately soluble in water. The intense staining with gold in the Bi-Col method (no other tissues in the body stain as intensely as this substance) indicates that it contains a very high concentration of weak acidic groups. The known fact that the parietal cells are acidophilic indicates that the net charge of these cells is positive; this probably implies that they contain numerous basic groups. It has been shown previously⁵ that the parietal cells stain intensely by treatment with OsO₄ in carbon tetrachloride, which might also indicate that they contain abundant hexamine moieties. From the solubility characteristics it may be surmised that the substance is not a lipid and from other tests that it contains little if any protein and is probably of a saccharidic nature.

Material of identical staining characteristics was found in the parietal cells of human and rat stomach when the specimens were fixed in Carnoy's fluid within a short time after death. In 2 instances of gastric achylia in patients with pernicious anaemia (one of them being in remission), no such substance was found in the glands of the body of the stomach.

It may be recalled that HOERR and BENSLEY⁶ reported on the presence of highly viscous material secreted by the parietal cells. It is surmised that the weak polyacid which stains with Bi-Col might be a precursor of HCl. The nature of the ion exchange which is probably involved in the secretion of HCl and the reason for the low organic content of parietal cell secretion need further study. A possible relationship between the weak polyacid and the intrinsic factor has not been investigated.

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Zusammenfassung

Parietalzellen aus Hundemagen sezernieren ein Polymer mit schwach sauren Gruppen, das mit der Bi-Col-Methode dargestellt werden konnte. Der Zusammenhang des Sekretes mit der Salzsäureproduktion wird diskutiert.

⁵ M. WOLMAN, Exp. Cell. Res. 12, 231 (1957).
⁶ N. L. HOERR and R. R. BENSLEY, Anat. Rec. 56, 417 (1936).

Über das Verhalten der optischen Isomeren von Adrenalin im Blut

Die teilweise Demethylierung, welche razemisiertes Adrenalin *in vivo* erfährt¹, und die unterschiedliche biologische Wirksamkeit seiner optischen Isomeren weisen auf ein von einander abweichendes Verhalten im Stoffwechsel hin.

Zur Abklärung dieser Frage wurden Katzen L- und D-Adrenalin infundiert und während der Infusion Adrenalin und Noradrenalin im Plasma fluorimetrisch bestimmt².

Diese Versuchsanordnung gestattet, die Abbaugeschwindigkeit des infundierten Stoffes zu messen und den Anteil Noradrenalin, der daraus gebildet wird, zu erfassen. Beim raschen Adrenalinabbau im Blut wird eine konstante Adrenalinkonzentration bereits nach wenigen Minuten erreicht (Plateau). Durch Demethylierung aus infundiertem Adrenalin gebildetes Noradrenalin erreicht wie dieses ein Plateau. Unphysiologisch hohe Adrenalinmengen wurden deshalb infundiert, um die Gleichgewichtskonzentrationen beider Pressoramine deutlich messbar über die Normalwerte zu heben. Gleichzeitig kann damit eine mögliche Grenze der Konzentrationsunabhängigkeit der Zerfallskonstanten ermittelt werden.

Das Verhältnis der Plateau-Konzentration von Noradrenalin zu derjenigen von Adrenalin ergibt den Anteil der Demethylierung (= Konversion) an allen Abbaureaktionen des Adrenalins unter der Voraussetzung gleicher Abbaugeschwindigkeiten für das primäre und das sekundäre Amin, wie sie in ¹ gefunden wurden.

Die Ergebnisse dieser Versuche, die 180 Einzelanalysen der beiden Pressoramine im Plasma erfassen, sind in den Tabellen I und II wiedergegeben.

Aus Tabelle I geht hervor, dass nach Infusion von L-Adrenalin die Konzentration von Noradrenalin im Plasma ansteigt, und zwar im Mittel auf mehr als den halben Wert des Adrenalinplateaus. Im Gegensatz dazu erhöht eine D-Adrenalinzufuhr die NoradrenalinKonzentration nicht (Tabelle II). Die Zerfallskonstanten λ sind für beide Isomere im Mittel dieselben; sie betragen etwa 0,5 min⁻¹, das heisst der Adrenalinabbau geht mit einer Halbwertszeit von durchschnittlich 80 s in diesen Infusionsversuchen vonstatten. Dieser Wert ist 2–3mal höher als das Mittel aus Abklingkurven und vorläufigen Dauerinfusionen.

¹ W. A. HUNZINGER, G. RITZEL und H. STAUB, Helv. chim. Acta 39, 2096 (1956).
² H. WEIL-MALHERBE und A. D. BONE, Biochem. J. 51, 311 (1952). – H. WEIL-MALHERBE und A. D. BONE, Lancet 1953, 974.

Tabelle I

Dauerinfusionen von L-Adrenalin an Katzen. Alle Konzentrationen sind auf Plasma bezogen. Noradrenalinwerte korrigiert für Analysendefizit

Katze Nr.	Infusion μMol/l min	Adrenalin μMol/l		Noradrenalin μMol/l		λ _L min ⁻¹	Konversion
		vor Infusion	Plateau	vor Infusion	Plateau		
3	0,44	0,016	0,51	0,027	0,48	0,86	0,9
4	0,375	0,003	0,57	0,017	0,098	0,65	0,1
5	0,515	0,003	0,84	0,060	0,23	0,61	0,2
11	0,65	0,011	1,5	0,018	0,36	0,44	0,2
12	0,345	0,024	0,79	0,086	0,38	0,44	0,4
13	0,63	0,012	3,8	0,016	4,5	0,17	1,2
14	0,58	0,013	1,2	0,13	0,77	0,50	0,6
15	0,24	0,020	0,60	0,095	0,73	0,40	1,2
Mittel:	0,47	0,013	1,22	0,056	0,94	0,51 ± 0,07	0,6 ± 0,16

Tabelle II
Dauerinfusionen von D-Adrenalin an Katzen
(übrige Angaben siehe Tabelle I)

Katze Nr.	Infusion $\mu\text{Mol/l min}$	Adrenalin $\mu\text{Mol/l}$		Noradrenalin $\mu\text{Mol/l}$		$\lambda_D \text{ min}^{-1}$	Konversion
		vor Infusion	Plateau	vor Infusion	Plateau		
6	0,67	0,005	0,71	0,033	0,084	0,95	0,07
7	1,25	0,024	2,7	0,060	< 0,018	0,46	0,00
9	0,95	0,007	2,1	0,035	0,018	0,44	0,00
10	1,00	0,015	2,5	0,031	< 0,018	0,41	0,00
16	0,43	0,035	0,90	0,073	0,064	0,48	0,00
17	0,47	0,005	0,77	0,046	0,060	0,61	0,02
18	0,63	0,024	2,6	< 0,015	< 0,018	0,25	0,00
19	0,58	0,007	0,84	0,055	< 0,018	0,68	0,00
Mittel:	0,75	0,015	1,64	< 0,043	< 0,037	$0,54 \pm 0,07$	$0,01 \pm 0,01$

Für die Pressoramine wurden die molaren Konzentrationen berechnet, weil dadurch die quantitativen Beziehungen zwischen Adrenalin und Noradrenalin direkt gegeben sind. λ = Zerfallskonstante des Adrenalins. Konversion = molares Verhältnis der Noradrenalin- zur AdrenalinKonzentration nach Einstellen des Gleichgewichtszustands (Plateau).

nen früherer Untersuchungen¹. Die Ursache hierfür kann in einer Abhängigkeit der Zerfallskonstanten von der zu-geführten Adrenalinmenge pro kg Körpergewicht liegen. Eine Regression von λ auf die pro Zeiteinheit und Körpergewicht infundierte Menge D- oder L-Adrenalin wurde in-dessen auch mit statistischen Mitteln nicht gefunden.

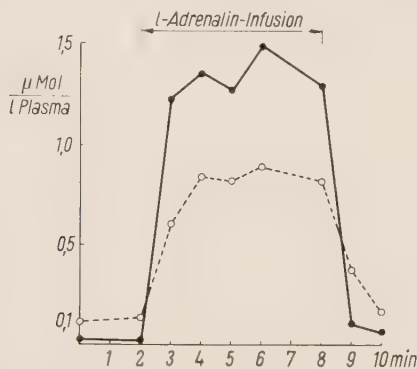


Abb. 1. L-Adrenalin-Dauerinfusion (Katze Nr. 14): Punkte = gefundene AdrenalinKonzentrationen; Kreise = gefundene NoradrenalinKonzentrationen; Konversion = 0,6; Infusion = $0,58 \mu\text{Mol/l min}$.

Das Verhältnis von Noradrenalin zu Adrenalin im Gleichgewichtszustand während der Infusion erweist sich als von der infundierten Adrenalinmenge unabhängig.

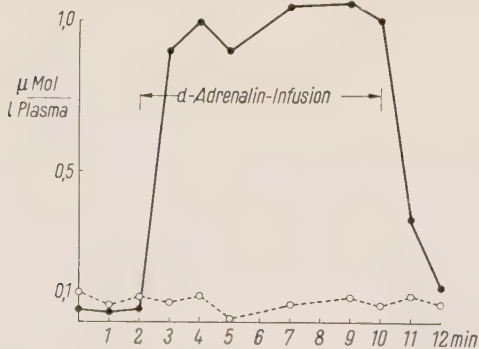


Abb. 2. D-Adrenalin-Dauerinfusion (Katze Nr. 16): Punkte = gefundene AdrenalinKonzentrationen; Kreise = gefundene NoradrenalinKonzentrationen; Konversion = 0,0; Infusion = $0,43 \mu\text{Mol/l min}$.

Zwei Einzelversuche sind in Abbildungen 1 und 2 wiedergegeben.

Versuche *in vitro* zeigen, dass zu Vollblut gegebenes Adrenalin mit der Zeit auf die Hälfte des Ausgangswertes absinkt. Die Geschwindigkeit dieses Vorgangs hat bei 38°C eine Halbwertszeit von etwa einer Stunde. Die kurze Dauer der Infusionen (10 bis 15 min) gestattet es, die quantitativen Auswirkungen der Adrenalinverteilung im Vollblut zwischen Plasma und Blutkörperchen unberücksichtigt zu lassen. (Über die Verteilung von Adrenalin zwischen Plasma und Erythrocyten siehe auch ³). Eine Umrechnung der im Plasma bestimmten Pressoramin-konzentrationen auf das entsprechende Blutvolumen fand daher bei den hier mitgeteilten Versuchen nicht statt.

Diskussion. Stoffwechseluntersuchungen von SCHAYER⁴ mit ^{14}C -markiertem Adrenalin am Ganztier haben ergeben, dass ein beträchtlicher Anteil der Methylgruppe oxydiert wird und als Kohlendioxyd in der Ausatemungs-luft erscheint. In β -Stellung markiertes C-Atom des Adrenalinmoleküls wird innert vier Stunden vollständig durch die Nieren ausgeschieden. Hieraus hat SCHAYER den Schluss gezogen, dass *in vivo* vom Adrenalinmolekül die Methyl- oder die Methylamino-Gruppe zu einem Teil abgespalten wird.

Nebennierengewebe andererseits kann unter Zusatz von Adenosintriphosphorsäure Noradrenalin zu Adrenalin methylieren⁵. Ergänzende Versuche am gleichen Substrat liessen die Umkehrreaktion, die Demethylierung von Adrenalin zu Noradrenalin, nachweisen⁶.

Die vorliegenden Versuche zeigen, dass sich während der L-Adrenalin-Infusion ein Gleichgewichtszustand zwischen Adrenalin- und NoradrenalinKonzentration rasch einstellt. Daraus lässt sich schliessen¹, dass im Stoffwechsel des L-Adrenalins die Demethylierung zu Noradrenalin ein erster Schritt sein muss. Neben dieser Reaktion müssen noch weitere Abbaumöglichkeiten vorhanden sein, da die Konversion zu Noradrenalin im Mittel aus acht Versuchen nur die Hälfte des gesamten L-Adrenalinabbaus ausmacht. Die Demethylierungsgeschwindigkeit (μ_1) verläuft mit einer Konstanten von

$$\mu_{1L} = 0,3 \text{ min}^{-1},$$

³ H. WEIL-MALHERBE und A. D. BONE, *Biochem J.* **58**, 132 (1954).
– W. A. BAIN, W. E. GAUNT und S. F. SUFFOLK, *J. Physiol.* **91**, 233 (1937).

⁴ R. SCHAYER, *J. biol. Chem.* **192**, 875 (1951).

⁵ E. BÜLBRING, *Brit. J. Pharmacol.* **4**, 234 (1949).

⁶ M. F. LOCKETT, *J. Physiol.* **117**, 68 P (1952).

wie sich auf einfache Weise aus der gefundenen Konversion (a) und der Konstanten des gesamten Abbaus (λ) errechnet:

$$\mu_{1L} = a \cdot \lambda.$$

Für D-Adrenalin fällt eine Demethylierung als Abbau-möglichkeit ausser Betracht ($\mu_{1D} = 0$).

Andererseits ergibt sich für λ_L und λ_D kein signifikanter Unterschied. Dies wäre nur durch eine Isomerieabhängigkeit der nicht in Transmethylierung bestehenden übrigen Abbaureaktionen zu erklären. Eine solche Isomerieabhängigkeit beim Metabolismus der Pressoramine wird schon durch den eindeutigen Unterschied in der Demethylierung von L- und D-Adrenalin offenbar. Auch andere, bisher besser bekannte Fermentreaktionen zeigen an stereoisomeren Substraten oft ausgeprägt asymmetrischen Verlauf.

Wir danken den Farbwerken Hoechst für die Überlassung von D-, L- und racemischem Adrenalin und Noradrenalin.

G. RITZEL, W. A. HUNZINGER
und H. STAUB

Medizinische Universitätsklinik Basel, 2. April 1958.

Summary

Demethylation of L- and D-adrenaline was studied on narcotized cats *in vivo*.

In metabolism of infused L-adrenaline, formation of noradrenaline represents one first step.

On the other hand, metabolism of D-adrenaline does not show any intermediate formation of noradrenaline.

Nachweis neuer Spurenkomponenten des Actinomycin-C-Komplexes

Zahlreiche Streptomyces-Stämme unserer Sammlung bilden bei der Kultivierung Actinomycin (Acm). Bisher konnten wir Acm B (X), C und D isolieren. Für die hier mitgeteilte Beobachtung verwandten wir Acm C, das von unseren Streptomyceten-Stämmen JA 1449 und JA 1521 gebildet wird.

Zur Gegenstromverteilung unserer Actinomycine, wobei wir eine in unserem Institut von H. BERG erstellte vollautomatische Craig-Apparatur benutzen konnten, sind neue Verteilungssysteme (Tab. I) – darunter erstmalig auch zwei salzfreie Drei- und Vierkomponenten-Systeme¹ – aufgefunden worden. Als besonders günstig für unsere Actinomycinproben erwiesen sich die Systeme 38/I und 43. System 43 (Tetrachlorkohlenstoff/4,5%ige wässrige Lösung von β -naphthalinsulfonsaurem Natrium) fiel bei der Systemsuche durch seine schnelle Gleichgewichtseinstellung, sein relativ gutes Lösungsvermögen für Acm, die günstige Lage des Mischverteilungskoeffizienten und seine sehr schnell verlaufende Phasentrennung nach dem Schütteln auf.

Nach der Craig-Verteilung von 100 mg Acm C über 184 Verteilungsschritte (Grundprozess) erhielten wir durch dieses System eine Verteilungskurve, die drei gut getrennte Maxima aufwies (Abb. 1). Die Auswertung der Ver-

teilung an Hand der Kurve und papierchromatographischer Messungen ergab folgende Befunde:

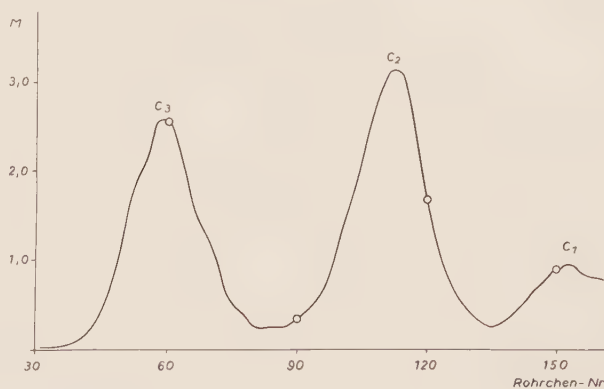


Abb. 1. Verteilungskurve von Acm C (System 43; $n = 184$; 1000 mg Acm C).

1. Die drei Maxima sind den Hauptkomponenten C_3 und C_2 und der Nebenkompente C_1 zuzuordnen. Ihre Verteilungskoeffizienten betragen $k_1 = 4,90$, $k_2 = 1,59$, $k_3 = 0,47$. Daraus ergeben sich die Trennfaktoren von $\beta_{1/2}^2 = 3,1$, $\beta_{2/3} = 3,4$ und $\beta_{1/3} = 10,4$.

2. Die vereinigten Fraktionen aus den Verteilungsröhrchen 25–51 zeigen neben der Hauptkomponente C_3 eine bisher nicht beobachtete neue Spurenkomponente, die wir C_{3a} nennen (Abb. 2 und 3).

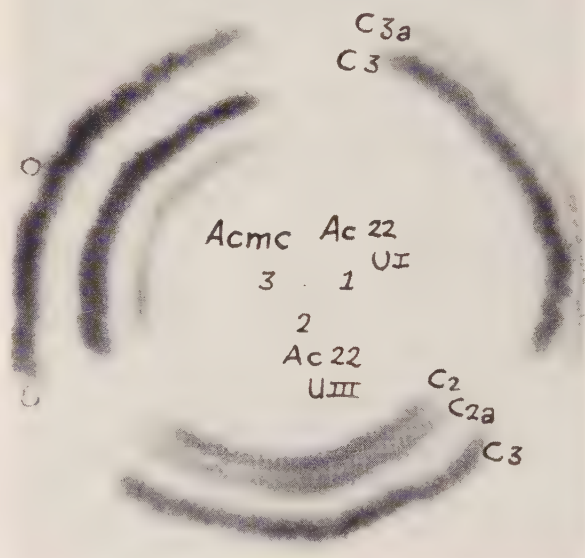


Abb. 2. Acm C mit den Spurenkomponenten $C_{2a} + C_{3a}$ in S 38/I.

3. Die Fraktionen aus den Röhrchen 70–100 (zwischen den beiden Hauptmaxima liegende Fraktionen) lassen neben den Komponenten C_3 und C_2 eine weitere Spurenkomponente des C-Komplexes erkennen, die wir als C_{2a} ³ bezeichnen (Abb. 2 und 3).

² $\beta_{1/2}$ bedeutet den Trennfaktor k_1/k_2 .

¹ Lediglich für die Papierchromatographie von Acm ist bereits von WAKSMAN ein salzfreies System (Cyclohexan-Methanol-Benzol-Propylenglykol 1:1:1:1) vorgeschlagen worden [Antibiot. Chemother. 5, 407 (1955)].

³ BROCKMANN *et al.* erwähnen in der Angew. Chem. 68, 70 (1956) ein Acm C_{2a} und verweisen dabei auf B. FRANCK, unveröffentlicht. Da keine Daten angegeben sind, ist es zur Zeit für uns nicht möglich, zu entscheiden, ob diese Komponente mit unserer identisch ist.

Tabelle I
Lösungsmittelsysteme für Actinomycin C

Systembezeichnung	Systemzusammensetzung	Anwendung zur
S 9	<i>n</i> -Dibutyläther- <i>n</i> -Butanol 5:1/10%ige wässrige Lösung von Thioharnstoff	PC
S 10	<i>n</i> -Dibutyläther- <i>n</i> -Butanol 5:1/2%ige wässrige Lösung von β -naphthalinsulfonsaurem Natrium	PC + SC Z
S 27/II	Cyclohexan-Amylacetat 1:1/5%ige wässrige Lösung von β -naphthalinsulfonsaurem Natrium	CCD
S 28	Cyclohexan-Amylacetat 4:6/5%ige wässrige Lösungen von β -naphthalinsulfonsaurem Natrium und β -Naphthalinsulfonsäure 1:1	CCD
S 34	Cyclohexan-Benzol 1:1/Methanol-Wasser 7:3	CCD
S 38/I	Isoamylacetat/5%ige wässrige Lösung von β -naphthalinsulfonsaurem Natrium	PC, CCD, SC Z
S 41	Tetrachlorkohlenstoff-Methanol-Wasser (100:80:20)	CCD
S 43	Tetrachlorkohlenstoff/4,5%ige wässrige Lösung von β -naphthalinsulfonsaurem Natrium	CCD
S 43/I	Tetrachlorkohlenstoff/2,5%ige wässrige Lösung von β -naphthalinsulfonsaurem Natrium	CCD

Erklärung: PC = Papierchromatographie;
CCD = Craig-Verteilung;
SC Z = Säulenchromatographie an Zellulose.



Abb. 3. Acm C mit den Spurenkomponenten C_{2a} + C_{3a} in S 10.

4. Da bei diesem System die wässrige (obere) Phase die mobile ist, resultiert – wie auch bei unserem System 41 – eine Umkehrung in der Wanderungsgeschwindigkeit der

Komponenten: C₃ («hydrophobe Komponente») wandert am langsamsten, C₁ («hydrophile Komponente») wandert am schnellsten.

Bei weiteren Verteilungen in diesem System, zum Teil mit geändertem Prozentgehalt an β -naphthalinsulfonsaurem Natrium, mit grösseren Acm-Einwagen und einer höheren Zahl von Verteilungsschritten (Grundprozess + laufende Entnahme der Oberphase) konnten wir jedesmal C_{2a} und C_{3a} papierchromatographisch nachweisen. Daneben waren – in noch weit geringerer Menge – zwei weitere Spurenkomponenten, C_{1a} und C₄, angedeutet. Beide gaben eben noch erkennbar – C_{1a} schwächer als C₄ – die typischen Farbreaktionen des Actinomycins mit Salzsäure und Zinn-II-chlorid.

In Tabelle II sind die R_{C2}-Werte der Actinomycin-C-Haupt-, Neben- und Spurenkomponenten in unseren Systemen 10 und 38/I aufgeführt. Setzt man die Identität des von BROCKMANN erwähnten C_{2a} mit dem hier genannten C_{2a} voraus, so ist beim Acm-C-Komplex mit neun Komponenten zu rechnen (C_{0a}, C₀, C₁, C_{1a}, C₂, C_{2a}, C₃, C_{3a} und C₄).

KH. ZEPF

Deutsche Akademie der Wissenschaften zu Berlin, Institut für Mikrobiologie und experimentelle Therapie, Jena, 14. Februar 1958.

Summary

After Craig distribution in our system 43 of actinomycin C, furnishing high β -values for the components of actinomycin, traces of new components of the actinomycin C complex could be demonstrated by paper chromatography.

Tabelle II
R_{C2}-Werte von Acm C in den Systemen 10 und 38/I

System		C ₀	C ₁	C _{1a}	C ₂	C _{2a}	C ₃	C _{3a}	C ₄
S 10	R _{C2} -Werte	0,2 ₅	0,69	0,8 ₁	1,0	1,15	1,39	1,56	1,7 ₅
S 38/I	R _{C2} -Werte	0,2 ₀	0,64		1,0	1,21	1,55	1,79	

Messungen bei 22–24°C; Papier: Schleicher und Schüll 2043 bmgl.

Paper Chromatographic Separation of some
Phloroglucinal Derivatives from *Dryopteris
filix mas*

From crude filicin, obtained via the oleoresin, filicic acid, flavaspidic acid and albaspidin were isolated, while filicinic acid was obtained by heating filicic acid in the waterbath with zinc dust and a 10% sodium hydroxyde solution for 6 h.

Paper. Use was made of Whatman No. 1 filter paper. The length of the employed strip was 270 mm and cut in the machine direction; the width was 70 mm.

Solvent. A 2 *N* sodium carbonate solution in which, immediately before chromatographing, 0.2 w/v% sodium sulfite had been dissolved.

Reagent. 1.5 ml of a solution of 1% sulfanilamide in 10% hydrochloric acid are mixed with 1.5 ml of a 5% sodium nitrite solution and gently shaken for 1 min. Next the whole partly neutralized with 1 ml 2 *N* sodium carbonate solution and water is added up to 50 ml. The reagent is always freshly prepared.

Ascending chromatography. 2 mg of the isolated substances are dissolved in 1 ml chloroform. By means of a pipette 3 µl of these solutions, are brought on the paper at 40 mm from the lower end of the strip at a mutual distance of 17.5 mm. In order to prevent difficulty in the spots being wetted by the solvent, first 1.5 µl is brought on the paper and after the chloroform has evaporated, 1 µl and finally 0.5 µl, instead of 3 times 1 µl. This is done to prevent the substance accumulating in the boundary of the spot.

When the spots are dry, the chromatogram is run at a temperature of 20°C. The paper is taken out of the cylinder as soon as the liquid front has reached a distance of 200 mm from the starting line, which takes about 3¼ h. Next the paper is dried for about 10 min in an oven at 80°C, sprayed with the reagent and dried again. Directly after the spraying the spots become visible and show some difference in colour for the different substances¹.

Result. See Table.

Substance	Colour	Average <i>R_f</i> value
Flavaspidic acid . . .	brownish-yellow	0.28
Filicic acid	brown	0.53
Albaspidin	violent-yellow	0.74
Filicinic acid.	orange-yellow	0.84

A mixture of these 4 substances shows the same average *R_f* values. The crude filicin itself, which is brought onto the paper in a concentration of 10 mg per ml chloroform, shows the *R_f* values of filicic acid and flavaspidic acid. In this concentration albaspidin is not visible. The crude filicin of some other fern species is being investigated.

S. GODIN

Pharmaceutical Laboratory of the State University (the Netherlands); Pharmacological Department, October 28, 1957.

Résumé

L'acide filicique, l'acide flavaspidique, l'albaspidine et l'acide filicinique ont pu être isolés par chromatographie sur papier, suivant la méthode ascendante, avec comme

phase mobile une solution de carbonate de sodium 2 *N*, dans laquelle on a dissous 2 g de sulfite de sodium par litre. Les spots étaient rendus visibles en pulvérisant une solution de sulfanilamide diazotée. En même temps se manifestait aussi une différence de couleur pour les dites substances phlorogluciniques.

The Influence of Oestrogen and Thyroid on
the Pituitary and Blood Content of FSH and LH

Hyperthyroidism when experimentally induced, increases the duration of the oestrous cycle, and this is attributed to prolongation of the luteal phase (HAYASHI¹ and WEICHERT²). Thyroid administration produced an increase in the number and size of corpora lutea (SIDKI³; WEICHERT and BOYD⁴ and SIDKI and SOLIMAN⁵). Such observations indicate that thyroid-active materials possess an oestrogen like effect by favouring the production of LH (STEIN and LISLE⁶; CHU⁷ and OKANS and TAKANA⁸).

Under natural conditions, the increased oestrogen level during the follicular phase of the oestrous cycle was revealed to be concomitantly associated with increased thyroid activity and increased level of thyrotrophic hormone in the blood (SOLIMAN and REINEKE⁹ and SOLIMAN and BADAWI¹⁰).

The present investigation was thus devised to ascertain the influence exerted by oestrogen and thyroid on the FSH and LH content of the blood and hypophyseal glands of ovariectomized rats.

Ovariectomy was performed on 24 mature female rats and these were divided into four groups with six animals in each. Group I was kept as control. To the rats of group II, desiccated thyroid was administered at the level of 0.1% in the diet for 30 days. Parenteral administration of 1 µg of oestradiol benzoate (B.D.H.) daily for 30 days was conducted to the animals of group III. The animals of group IV were treated with oestradiol and, in addition, desiccated thyroid was fed for the same duration, after which all the animals were killed by decapitation under light ether anesthesia, and the blood of the animals of each group was pooled together and allowed to coagulate. The sera were procured by centrifugalization at a rate of 3,000 r.p.m. for 15 min and subsequently treated with acetone and ethyl alcohol in order to precipitate the proteins and the precipitate that arose was redissolved in saline so as to bring the sera back to their original volume. Such a procedure was adopted to annul any error that might arise from the presence of oestrogen in the serum of the rats. The hypophyseal glands of each group were cleanly dissected, weighed in a torsion balance, pooled together and thoroughly ground. The resulting suspension was so adjusted that every 0.1 ml of saline contained 1 mg of fresh hypophysis.

1 H. HAYASHI, Bull. Acad. Med. (Paris) 101, 115 (1929).
2 C. K. WEICHERT, Physiol. Zool. 3, 461 (1930).
3 Y. SIDKI, Interrelationship of Endocrine Organs (University of Edinburgh, 1933).
4 C. K. WEICHERT and R. W. BOYD, Anat. Rec. 58, 55 (1933).
5 Y. SIDKI and F. A. SOLIMAN, Egypt. Vet. Med. J. 3, 117 (1956).
6 K. F. STEIN and M. LISLE, Endocrinology 39, 16 (1942).
7 J. P. CHU, Endocrinology 34, 90 (1944).
8 K. OKANS and H. TAKANA, Trans. Soc. Path. (Japan) 30, 239 (1946).
9 F. A. SOLIMAN and E. P. REINEKE, Amer. J. Physiol. 178, 89 (1954).
10 F. A. SOLIMAN and H. M. BADAWI, Nature 172, 235 (1956).

¹ The spraying with reagent is done in the fume-chamber.

Table I
Influence of oestrogen and thyroid on FSH content rat blood and pituitaries

Treatment of Rats	Ovary Weights of Mice in mg/100 g Body Weight	
	Blood	Pituitary
Castrated	76.52 $\pm$ 3.22	87.80 $\pm$ 2.25
Castrated + Thyroid	62.78 ^a $\pm$ 2.68	72.07 ^b $\pm$ 2.48
Castrated + Oestradiol	65.47 ^a $\pm$ 3.60	62.05 ^b $\pm$ 1.69
Castrated + Oestradiol + Thyroid	49.32 ^b $\pm$ 1.70	47.75 ^b $\pm$ 1.13
Control Mice	17.25 ^b $\pm$ 1.02	

$\pm$ Standard error.

^a Significantly different from castrated rats at 1% level.

^b Significantly different from castrated rats at 5% level.

The FSH content of 0.5 ml of serum or 1 mg of hypophyseal substance was determined by the LH augmentation method adopted by BROWN¹¹. The above-mentioned amounts, together with 20 I.U. of chorionic gonadotrophin, were parenterally administered into 21-day-old female mice. 54 animals were utilized for this purpose and these were subdivided into 9 groups of 6 animals each. One group was retained as control and two of the remaining groups were allotted to each of the experimental groups, one set receiving serum while to the other hypophyseal suspension was administered.

The LH content, on the other hand, was determined by following the method adopted by SOLIMAN¹², in which groups of 5 immature female mice were primarily treated with 20 I. U. of pregnant mare serum, parenterally given. After 4 days when the graafian follicles attained full development, 0.5 ml serum or 1 mg hypophyseal substance was injected into the mice. The animals were then killed with ether 48 h later, and the number of the corpora haemorrhagica formed was considered as an index of the LH content of the test material.

As Table I shows, the FSH content of the blood and pituitaries of ovariectomized rats were the largest. Administration of either oestrogen or thyroid to ovariectomized rats induced a drop in the FSH content of both their blood and pituitaries, presumably due to decreased rate of formation and release of the hormone by the pituitaries. The combined administration of oestrogen and thyroid caused a further decrease in the level of FSH in the blood and pituitaries.

It appears from Table II that administration of oestrogen and thyroid, singly or in combination, significantly increased the concentration of LH in the blood of the experimental animals. The level of LH, however, was

greater on joint administration of the two hormones than when either of them was given separately. Oestrogen administration was accompanied with a pronounced increase in hypophyseal LH. Thyroid administration caused a mild increase in pituitary LH. It appears, then, that oestrogen favours the synthesis of LH by the pituitaries while thyroid administration increases the rate of release of this hormone. The LH content of the pituitaries of rats treated with the two hormones together was less than that of oestrogen-treated animals.

In the light of the data obtained from the present investigation, it is apparent that ovulation is associated with thyroid activity. Under natural conditions, there is an increased production of both oestrogen and thyroid hormones during oestrus which induces a drop in the FSH level in the blood with a prompt rise in the level of LH, a phenomenon that induces ovulation.

Thanks are due to Mr. ABD-EL-WAHAB S. AHMED of the United States Medical Research Unit No. 3, Cairo, Egypt, for supplying us with the mice used in this investigation.

Y. SIDKI, H. M. BADAWI, and
F. A. SOLIMAN

Department of Physiology, Faculty of Veterinary Medicine, Cairo University, Giza (Egypt), March 13, 1958.

Résumé

L'effet de l'administration du «Benzoate d'oestradiol» et des hormones thyroïdales seuls ou en combinaison sur le FSH et LH contenus dans le sang et les hypophyses des femelles rats castrés a été étudié. Il a été trouvé que l'oestradiol et l'extract de la thyroïde quand ils sont administrés seuls produisent une baisse, dans le sang, du FSH et une hausse du LH. L'administration combinée de ces deux hormones renforce cet effet.

Table II
Influence of oestrogen and thyroid on LH content of rat blood and pituitaries

Treatment of Rats	Average No. of corpora hemorrhagica	
	Blood	Pituitary
Castrated	0.50 ^b $\pm$ 0.02	1.20 $\pm$ 0.09
Castrated + Thyroid	1.80 ^b $\pm$ 0.01	2.00 $\pm$ 0.35
Castrated + Oestradiol	2.00 ^b $\pm$ 0.01	2.50 ^b $\pm$ 0.33
Castrated + Oestradiol + Thyroid	2.40 ^b $\pm$ 0.11	1.40 $\pm$ 0.11
Control Mice (20 I. U., PMS)	0.33 $\pm$ 0.10	—

$\pm$ Standard error.

^b Significantly different from castrated rats at 1% level.

Über die mögliche Bedeutung von pH-Unterschieden für den Transport von höheren Fettsäuren im Organismus

Während einer durch Heparininjektion verursachten Klärungsreaktion wird das lipolytische System des Blutes in hohem Grade aktiviert¹. Dabei entstehen unveresterte Fettsäuren (UFS), die jedoch die Blutbahn rasch wieder verlassen; ihr Abtransport erfolgt, wie aus unseren Experimenten am Hund hervorgeht, mit einer Durchschnittsgeschwindigkeit von 0,3 mÄq/l Plasma/min².

Im Blutstrom sind die UFS vollständig oder wenigstens beinahe vollständig³ an die Bluteiweisskörper gebunden, und zwar am stärksten an das Albumin. Vorläufig wurde noch nicht bewiesen, dass ein UFS-Albumin-Komplex den Blutstrom verlässt. Doch wurde schon wenige Minuten nach Verabreichung von mit ¹³¹I markiertem Albumin dieses in der Lymphe nachgewiesen⁴; es ist somit sicher, dass Albumin rasch aus der Blutbahn austreten kann. Neueste Arbeiten ergeben dafür eine Geschwindigkeit von 5 g Albumin/h⁵.

Da Albumin höhere Fettsäuren sehr stark bindet, kann angenommen werden, dass auch im extravasalen Teil der extrazellulären Flüssigkeit, in dem sich 60% des austauschbaren Albumins befinden⁶, die UFS an diesen Eiweisskörper gebunden sind. Auch ist bekannt, dass Fettsäuren imstande sind, aus einem Albumin enthaltenden Substrat in die Zellen einzudringen⁷. Ferner wurde für einige Farbstoffanionen bewiesen, dass sie gemeinsam mit dem Albumin in das Zellinnere eindringen⁸. Der Mechanismus, der die UFS aus ihrem Komplex mit dem Albumin wieder abtrennt, ist bis jetzt nicht bekannt. Eine Reversion der Albumin-UFS-Bindung könnte dadurch eintreten, dass die Fettsäuren an einem Eiweisskörper der Mitochondrien fester gebunden werden als an das Albumin, wie dies für einige Farbstoffe bereits bewiesen wurde⁹.

Nach SAROFF¹⁰ nimmt mit sinkendem pH die Bindungsfähigkeit des Albumins für höhere Fettsäuren ab. Während das extrazelluläre pH 7,4 beträgt, liegt das intrazelluläre pH bedeutend niedriger und kann einen Wert von 5,3 erreichen¹¹. Die Bedeutung dieser Tatsachen für den Transport von Fettsäuren wurde in einem Modellversuch geprüft. Dazu untersuchten wir die Verteilung von Oleat zwischen Albuminlösungen verschiedener Konzentration mit pH 7,4 bzw. 5,3 einerseits und Olivenöl, das in diesen Experimenten die Zellfettkomponenten repräsentierte, andererseits. Im wesentlichen handelt es sich also um eine Verteilung zwischen zwei Phasen, wie sie von KARUSH¹² für die Untersuchung der Albumin-Methylorange-Bindung verwendet wurde.

Experimenteller Teil. Das Albumin war ein elektrophoretisch einheitliches, menschliches Serumalbumin (Fraktion V) und wurde jeweils gegen den entsprechenden

Albuminkonzentration in %	UFS-Konzentration in mÄq/l					
	pH 7,38			pH 5,29		
	Anfang	Ende	Unterschied	Anfang	Ende	Unterschied
1	1,97	1,35	— 0,62	1,99	1,01	— 0,98
	2,00	1,35	— 0,65	1,97	0,94	— 1,03
	1,99	1,38	— 0,61	1,98	0,97	— 1,01
2	2,01	1,75	— 0,26	2,02	1,42	— 0,60
	2,01	1,79	— 0,22	2,00	1,39	— 0,61
	2,01	1,72	— 0,29	2,01	1,40	— 0,61
3	1,95	2,30	+ 0,35	1,95	1,48	— 0,47
	1,99	2,29	+ 0,30	1,98	1,52	— 0,46
	1,97	2,31	+ 0,34	1,97	1,49	— 0,47
4	1,95	2,58	+ 0,53	1,98	1,65	— 0,33
	1,99	2,61	+ 0,62	2,02	1,61	— 0,41
	1,98	2,59	+ 0,61	2,00	1,68	— 0,32

Sörensen-Puffer (0,15 M) dialysiert. Nach der Dialyse wurde die Albuminkonzentration korrigiert; die dabei benötigten Analysen wurden nach der Biuret-Methode vorgenommen. Das Oleat war ein p.A.-Präparat der Firma Merck. Die Säurezahl des Olivenöls betrug 0,323.

In einem Vorversuch wurden 4 ml einer 2,5-mÄq/l-Oleatlösung im Puffer von pH 7,4 ohne Albumin im Scheidetrichter mit 6 ml Olivenöl überschichtet. Die wässrige Phase wurde aus den einzelnen Scheidetrichtern in verschiedenen Zeitabständen entfernt, davon 1 ml abpipettiert und darin die UFS nach der Doleschen Methode³ bestimmt. Dabei fanden wir, dass sich die UFS-Konzentration einer auf pH 7,4 gepufferten, mit Olivenöl überschichteten 2,5-mÄq/l-Oleatlösung in 40 h praktisch nicht mehr ändert. Deshalb wurden die UFS-Konzentrationsunterschiede in der auf pH 7,4 bzw. 5,3 gepufferten, wässrigen Phase in Gegenwart von Albumin nach 48 h gemessen. Die UFS-Anfangskonzentration betrug durchwegs 2 mÄq/l. In der Tabelle sind die Untersuchungsergebnisse zusammengefasst. Es zeigt sich, dass die Verteilung des Oleates in Lösungen von pH 7,4 mit grösserer Albuminkonzentration als 2% zugunsten der wässrigeren Phase ausfällt, während das Oleat aus Lösungen mit pH 5,3 sogar bei einer Albuminkonzentration von 4% in die Ölphase eindringt.

Wahrscheinlich macht sich bei dieser Methode auch die Adsorption an der Wasser-Öl-Grenzfläche geltend, wie sie für eine Albumin-Dodecylsulfat-Wasser-Öl-Emulsion von COCKBAIN¹³ beschrieben wurde; doch kann dies in Anbetracht der geringen Grenzfläche vernachlässigt werden.

Diese Versuche liefern ein Modellanalogon möglicher Vorgänge, wie sie sich beim Fettsäuretransport in das Zellinnere abspielen könnten. Aus den Resultaten ergibt sich, dass der Unterschied zwischen intra- und extrazellulärem pH ein Faktor sein dürfte, der den Fettsäuretransport in das Zellinnere ermöglicht.

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Summary

The distribution of oleate between buffered albumin solutions (albumin concentration 1, 2, 3 and 4%) pH 7.4 and 5.3, and olive oil has been investigated. The possible significance of the findings for the transport of fatty acids from the extracellular fluid (pH 7.4) into cells, where the pH is more acid, is discussed.

¹³ E. C. COCKBAIN, Trans. Faraday Soc. 49, 1 (1953).

¹ A. V. NICHOLS, N. K. FREEMAN, B. SHORE und L. RUBIN, Circulation 6, 437 (1952). — R. K. BROWN, E. BEYLE und C. B. ANFENSEN, J. biol. Chem. 204, 423 (1953).

² V. FELT, D. REICHL und D. GRAFNETTER, Čs. Gastroenterologie 12, 153 (1958).

³ V. P. DOLE, J. clin. Invest. 35, 150 (1956).

⁴ H. KRIEGER *et al.*, Proc. Soc. exp. Biol. Med. 73, 124 (1950).

⁵ S. A. BERSON, Fed. Proc. 16, Suppl. 1 (1957).

⁶ S. A. BERSON *et al.*, J. Clin. Invest. 32, 746 (1953).

⁷ I. STERN und B. SHAPIRO, Metabolism 3, 539 (1954).

⁸ H. BENNHOLD, Acta med. scand. Suppl. 312 (1955).

⁹ E. KALLEE, Arch. Biochem. Biophys. 60, 265 (1956).

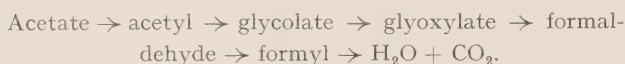
¹⁰ A. F. SAROFF, siehe R. S. GORDON *et al.* Proc. Soc. exp. Biol. Med. 84, 168 (1953).

¹¹ E. J. CONWAY und P. J. FEARON, J. Physiol. 103, 274 (1944).

¹² F. KARUSH, J. Amer. chem. Soc. 72, 2705 (1950).

Possible Relations Between the Direct Oxidation System of Acetate (MAS) and the Tricarboxylic Acid Cycle (TAC) in Experiments with Living Yeast Cells

The existence of a direct way of acetate oxidation in yeast¹ and in *E. coli*² has been demonstrated according to the following scheme:



All intermediates have been isolated. In experiments with yeast and labeled acetate, the distribution of radioactivity in the intermediates was in accord with the reported scheme³. It seemed of interest to establish whether the two systems occur simultaneously in the yeast cells by determining in the medium the TAC intermediates which may be present besides those of the MAS.

In fact paper chromatographic analysis have shown the presence of succinic, fumaric, malic and citric acid together with the C₂ and C₁ intermediates of the MAS, usually present. This report deals with the identification of these acids and the possible relations between the TAC and the MAS are discussed.

Details of the procedure used in the experiments and for the isolation of the intermediates of the MAS were described in previous papers⁴.

The acetyl and the formyl were calculated respectively from the acetophenone and the benzaldehyde formed⁵. In the experiments of acetate oxidation without phenylhydrazine (blank test), the pH was maintained around 7 by dropwise addition of 10% sulphuric acid solution for at least 2 h. The separation of the di- and tricarboxylic acids from the medium was performed according to the method outlined for the glycolic acid⁶, the time of the ether extraction lasting for 50–60 h, instead of 18–20 h. The conventional descending technique of BRYANT and OVERELL⁶ for the paper chromatographic identification of the acids was used. Citric acid was determined by the method of TÄUFEL *et al.*⁷, glycolic acid by the method of DAGLEY *et al.*⁸. Succinic, fumaric and malic acids were detected by visual comparison of the intensity of colour and the size of the spots obtained with those of standard solutions.

The results are summarized in the Table. It is interesting that the acids of the TAC and the glycolic acid of the MAS are simultaneously present in the experiments of acetate oxidation without phenylhydrazine, i.e. under normal physiological conditions. Since the same result is almost obtainable in the presence of phenylhydrazine,

Intermediates found in the medium during acetate oxidation with and without phenylhydrazine. Average results of four experiments.

Intermediates	Incubation without phenylhydrazine	Incubation with phenylhydrazine
	mg/l	mg/l
Acetyl	0.0	45.0
Formaldehyde	0.0	2.0
Formyl	0.0	4.0
Glycolic acid	1.5	3.0
Succinic acid	15.0	11.0
Fumaric acid	0.0	2.0
Malic acid	4.5	2.0
Citric acid	2.5	1.0

it may be concluded that this product has no or weak toxic action on the enzymes of the TAC.

The presence in the medium of the intermediates of the MAS besides those of the TAC (or also of the DAC) suggests that two acetate oxidation systems occur simultaneously in yeast cells. This result is consistent with the view already expressed by KREBS *et al.*⁹: 'That two mechanisms may occur in the same species side by side, the relative preponderance varying according to environmental conditions'. Recent findings of WIAME and BOURGEOIS¹⁰, McQUILLEN and ROBERTS¹¹ and KATZ and CHAIKOFF¹² in accord with KREBS *et al.*⁹ ascribe to the TAC energetic and synthetic functions. It is possible that this double function may also be assigned to the MAS. Probably, within the microbial cells more than one oxidative system occurs, each system having the purpose to produce some (or specific) intermediates for microbial synthesis. Therefore we suppose that the TAC supplies C₄, C₅ and C₆ fragments and the MAS C₁ and C₂ fragments simultaneously with the energy necessary for their utilization.

Furthermore we suppose that the isocitritase of yeast¹³, and the malate synthetase of WONG and AJL¹⁴, or a similar enzyme, may be the enzymes able to establish relations between the TAC and the MAS. In fact, isocitritase by splitting isocitrate into succinate and glyoxylate permits the passage from an intermediate of the TAC to another of the MAS and *vice versa*. The same observation accounts for the malate synthetase which synthesizes malate from acetate and glyoxylate.

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Zusammenfassung

Es wird nachgewiesen, dass in der Hefe die Oxydation des Azetates sowohl nach dem Krebs-Zyklus als auch direkt über Glykolsäure, Glyoxylsäure und Formaldehyd ablaufen kann. Dabei kommen Isozitroneinsäure-Lyase und Apfelsäure-Synthetase als Brücke zwischen den beiden Atmungssystemen in Frage.

⁹ H. A. KREBS, S. GURIN, and L. EGGLESTON, *Biochem. J.* **51**, 614 (1952).

¹⁰ J. M. WIAME and S. BOURGEOIS, *Nature* **172**, 310 (1953). – J. M. WIAME, *Adv. Enzymol.* **18**, 241 (1957).

¹¹ K. McQUILLEN and R. B. ROBERTS, *J. biol. Chem.* **207**, 81 (1954).

¹² J. KATZ and I. L. CHAIKOFF, *Biochim. biophys. Acta* **18**, 87 (1955).

¹³ J. A. OLSON, *Nature* **174**, 695 (1954).

¹⁴ D. T. O. WONG and S. J. AJL, *J. Amer. chem. Soc.* **78**, 3230 (1956).

¹ V. BOLCATO, B. DE BERNARD, and G. LEGGIERO, *Arch. Biochem. Biophys.* **69**, 372 (1957). – V. BOLCATO and M. E. SCEVOLA, *Naturwissenschaften* **44**, 539 (1957). – V. BOLCATO and G. LEGGIERO, *Ann. chimica* **48**, 177 (1958).

² V. BOLCATO, E. BOSCHETTI, and E. MONTOYA, *Ant. v. Leeuwenhoek* **22**, 131 (1956).

³ V. BOLCATO, B. DE BERNARD, and G. LEGGIERO, *Arch. Biochem. Biophys.* **69**, 372 (1957).

⁴ V. BOLCATO, B. DE BERNARD, and G. LEGGIERO, *Arch. Biochem. Biophys.* **69**, 372 (1957). – V. BOLCATO, M. FRASCHINI, and G. BONI-PERTI, *Ant. v. Leeuwenhoek* **22**, 419 (1956).

⁵ V. BOLCATO and M. E. SCEVOLA, *Naturwissenschaften* **44**, 539 (1957).

⁶ F. BRYANT and B. T. OVERELL, *Biochim. Biophys. Acta* **10**, 471 (1953).

⁷ K. TÄUFEL, R. POHLONDEK-FABINI, and U. BEHNKE, *Z. anal. Chem.* **146**, 244 (1955).

⁸ S. DAGLEY and A. RODGERS, *Biochim. biophys. Acta* **12**, 591 (1953).

Effects of Thyrotropin and Thiouracil on Embryonic Chick Thyroid *in vitro*

It was reported in previous accounts that organ rudiments of the chick embryo develop satisfactorily on a medium consisting solely of adult tissue derivatives¹. In further experiments on these lines a procedure has been evolved by us in which the use of heterologous adult tissue derivatives as the liquid culture medium and a cellulose membrane as the mechanical support for the explants have been introduced². This procedure is but a modification of Fell's classical watch-glass technique for the cultivation of embryonic organ rudiments³.

In the present experiments the embryonic chick thyroid was chosen for cultivation with the liquid medium-cellulose membrane culture procedure.

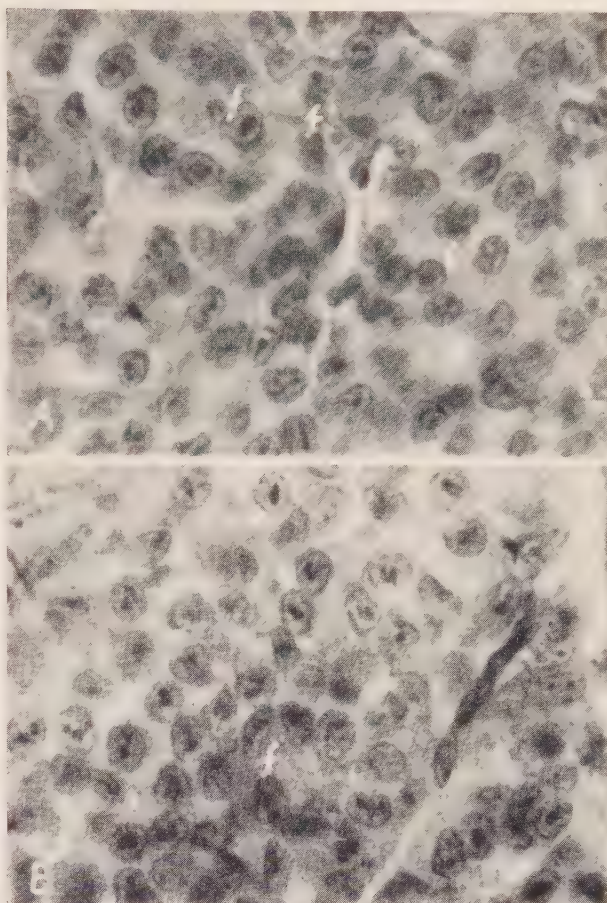
The development *in vitro* of the embryonic chick thyroid was described by CARPENTER⁴. GAILLARD⁵ reported on the effect of thyrotropic hormone on such thyroids in culture, advancing the assumption that the formation of 'colloid' and that of follicles may be dissociated processes. Therefore, explanted thyroid rudiments were subjected to the influence of thyrotropic hormone and thiouracil and their development as regards 'colloid'- and follicle-formation under such conditions was studied.

Results. 20 cultures of thyroids from 8-day chick embryos were grown on the horse serum-ascitic fluid medium. After 2 days *in vitro* droplets of 'colloid' were found scattered throughout the explant. It is difficult to define their location as being intra- or intercellular in position, since histologically the tissue, at this stage, is a symplasmatic structure without cell borders⁶. However, some 'colloid'-droplets appeared surrounded by 3–5 nuclei, the structure suggesting strongly small follicles. After additional 2 days of cultivation follicles were formed, which consisted of 7–9 cells in section, and were filled with intrafollicular 'colloid'. Up to 8 days *in vitro* – after which period the cultures were discontinued – the number of follicles increased steadily.

The series of cultures, in which the effects of thyrotropin (Ambinon, Organon; 0.01 U) and thiouracil (0.4 γ) were studied, was arranged as follows: Both lobes of one embryonic thyroid were cultivated each with a different agent. Thus possible individual differences were excluded and the cultures could be compared as to the effects of the agents. One lobe was grown on a medium consisting of 5 drops of horse serum, 2 of ascitic fluid and 1 drop of Ambinon. The second was cultivated on a similar medium in which 1 drop of thiouracil was substituted for that of Ambinon. In total 36 cultures were grown and examined 18 of each type.

In the cultures containing Ambinon the follicles, formed after 2 days *in vitro*, were considerably larger and comprised more cells than those seen after the same period in explants on horse serum-ascitic fluid medium. They were comparable to those which formed on the latter medium only after 3–4 days in culture, since they consisted of 5–8 cells in section, and were filled with 'colloid'. After 4 days

in vitro there were numerous large follicles in the explant, more than in any other culture examined (Fig. A).



Sections through 8-day thyroid rudiments after 4 days' cultivation: A on a medium containing Ambinon, showing many large follicles filled with 'colloid'; B, on a medium with thiouracil, showing small follicles, devoided of 'colloid'; f = follicle. H.-E., $\times 1140$.

In cultures containing thiouracil after 2 days neither follicles nor 'colloid' droplets were discernable. Only after 4 days *in vitro* very few small follicles appeared in the explants (Fig. B). When compared to the cultures on Ambinon and those grown on horse serum-ascitic fluid only, these explants were greatly retarded with regard to both 'colloid' and follicle formation. Although on the 7th to 8th day of cultivation the differences mentioned seem to become less pronounced, the formation of typical intrafollicular 'colloid' was not evident even at this stage.

Comment. In the thyroid of 8-day chick embryos grown on the horse serum-ascitic fluid medium the first follicles appeared after two days. In comparison with the normal gland the onset of follicle formation was one day earlier. The influence of thyrotropic hormone revealed itself in the early differentiation of follicles, and in their large size at the onset of their formation. The effect of thiouracil was evident in the much delayed differentiation of the follicles and in the almost complete arrest of 'colloid' formation. The differences in development of the embryonic thyroid on various culture media were most conspicuous after the 4th day. The thyrotropin exerted its influence on the onset of the 'colloid' formation rather, than on the follicle development. Thiouracil, on the other hand, retarded both the 'colloid' and follicle formation.

¹ H. MOSCONA (SOBEL) and A. MOSCONA, J. Anat., Lond. 86, 278 (1952). – H. SOBEL and A. MOSCONA, Exper. 10, 502 (1954).

² A. MOSCONA and H. MOSCONA (SOBEL), Bull. Res. Council. Israel 3, 197 (1953).

³ H. B. FELL, J. Roy. micr. Soc. 60, 96 (1940).

⁴ E. CARPENTER, J. exp. Zool. 89, 407 (1942).

⁵ P. J. GAILLARD, Versl. gwone Vergad. Akad. Amst. afd. Nat. 61, 27 (1952).

⁶ W. BRADWAY, Anat. Rec. 42, 157 (1929). M. L. HOPKINS, J. Morph. 58, 585 (1935).

These findings corroborate GAILLARD's conclusion that the formation of 'colloid' and that of follicles in the embryonic thyroid are dissociated processes⁵. They suggest, moreover, that the thyrotropic and antithyroid agents can exert their respective effects on the embryonic gland *in vitro* already during the stage of histological differentiation. The agents used affected the formation of 'colloid' more markedly than that of follicles.

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Zusammenfassung

Schilddrüsenanlagen von Hühnerembryonen, in Ambion-Medium kultiviert, entwickeln sich schneller und zeigen beschleunigte intrafollikuläre Kolloidbildung. Mit Thiourazil behandelte Kulturen liessen retardierte Follikelbildung und Kolloidbildung erkennen.

The Organic Acid-Soluble Phosphate Contents of Mammalian and Avian Erythrocytes at the Beginning of Postnatal Life

In a previous communication¹ it has been pointed out that the organic acid-soluble phosphate (OAS-P) level in the erythrocytes of the newborn dog and rabbit is very low in comparison to that in the adult animal. This is a finding which is not at all in accord with observations reported by other investigators in various animal species. All the data in the literature available to us indicate that age and the OAS-P contents of the cellular elements correlate just the other way round. The red blood cells of the lamb², the calf³, and the young rat⁴, are richer in components containing OAS-P than the adults of these species. It is on this basis that GUEST and RAPOPORT⁵ regard the relatively higher organic phosphate level of the erythrocytes as characteristic of the young of all mammals. With a view to elucidating the prevailing contradictions our analyses were extended to numerous mammalian species and a number of domestic birds. The present paper incorporates the results obtained.

The organic phosphate fractions were determined from whole blood in the main according to BOMSKOV's method⁶. The corresponding phosphate levels of the red cells were calculated on basis of the hematocrit values. The so-called resistant fraction ($P_{\text{acid soluble}} - P_{180}$) contains the phosphoglycerate-phosphates of the blood. Except for the ruminants, in the red blood cells of the mammals the overwhelmingly greater part of the fraction consists of 2,3-diphosphoglycerate. Simultaneously with the working up of the blood of the newborns, determinations were in every case made from adult animals as well. Since the data we obtained for them agreed largely with the data in the literature, we were content to use a relatively small number of adults. Our findings, presented numerically in the attached Table, appear to permit the following conclusions.

1 F. KUTAS and M. STÜTZEL, Acta vet. hung. 8, 1 (1958).
2 A. I. MALAN, J. agric. Sci. 18, 397 (1928).
3 H. H. GREEN and E. H. MACASKILL, J. agric. Sci. 18, 384 (1928).
4 S. RAPOPORT and G. M. GUEST, J. biol. Chem. 126, 749 (1938).
5 G. M. GUEST and S. RAPOPORT, Phys. Rev. 21, 410 (1941).
6 C. BOMSKOV, Hoppe-Seylers Z. 210, 67 (1932).

The average values of OAS-P and resistant P in the erythrocytes of newborn and adult animals

Ordo	Spécies	Group ¹	Number of animals	OAS-P ($P_{\text{ac. sol.}} - P_{\text{inorg.}}$)	Resist. P ($P_{\text{ac. sol.}} - P_{180}$)
				mg/100	ml cells
Ungulata . . .	Horse	N	4	51.05	39.58
		A	3	51.87	42.17
	Pig	N	9	75.19	35.53
		A	5	102.62	72.30
	Cattle	N	5	42.98	27.30
		A	4	12.05	5.13
	Sheep	N	6	57.77	29.17
		A	3	20.87	3.47
	Goat	N	2	58.85	33.25
		A	2	15.33	6.20
Carnivora . . .	Dog	N	14	34.71	13.03
		A	5	53.42	40.48
	Cat	N	10	19.16	10.00
		A	3	21.17	14.40
Rodentia . . .	Rabbit	N	20	42.68	16.68
		A	6	80.48	53.23
	Guinea pig	N	6	36.20	26.33
		A	3	49.30	36.20
Primates . . .	Man	N	3	48.30	30.90
		A	3	48.03	33.83
Rasores . . .	Chicken	N	10	90.97	—
		A	3	95.73	—
	Turkey	N	4	119.75	—
Lamellirostres .	Duck	A	3	98.23	—
		A	3	108.43	—
			4	116.20	—

¹ N = Newborn, A = Adult

(1) From the point of view raised in the introduction, the mammals we studied can be divided into 3 groups. In 4 species (dog, rabbit, pig, and guinea pig), the red blood cells contain substantially less OAS-P in the newborn than in the adult animal. With the ruminants (cattle, sheep and goat) the situation is just the reverse: in relation to those of the adults, the erythrocytes of the newborns are remarkably rich in OAS-P. The rat too can be classed with this group. Finally, we have man, the dog and the cat, who show no appreciable differences in the two age groups.

The average values for domestic birds agree in the various age groups.

(2) The data in the Table show that from among the organic acid-soluble phosphate compounds, it is the phosphoglycerates (belonging to the resistant fraction), that are responsible for any differences, for the resistant P contents invariably change with the changes in the OAS-P level. Accordingly, in nucleated erythrocytes devoid of phosphoglycerate (avian), the OAS-P level is practically the same in the two age groups.

(3) As early as 1898, considerable differences were described by ABDERHALDEN⁷, in the phosphate contents of the blood of various mammalian species. Since the studies of RAPOPORT and GUEST⁸, the fraction which shows the widest quantitative differences by species, is known to be formed by the phosphoglycerates. Contrary to these conditions prevailing in adult animals, in the red cells of newborn mammals differences according to species of a substantially lower degree were only observed by us. Particularly striking is the great similarity of the resistant

7 E. ABDERHALDEN, Hoppe-Seylers Z. 25, 65 (1898).
8 S. RAPOPORT and G. M. GUEST, J. biol. Chem. 138, 269 (1941).

phosphate contents within the individual mammalian orders (Ungulata, Carnivora, Rodentia). In red blood cells produced in the earlier (foetal) ontogenetic stage the specific differences are assumedly even less distinct.

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Institute of Physiology, Veterinary Faculty, Budapest, February 2, 1958.

Zusammenfassung

Die bisherigen Angaben eines reichen organischen säurelöslichen Phosphatgehalts der Erythrozyten können für junge Tiere nicht verallgemeinert werden.

Bei zahlreichen Säugerarten zeigen die Erythrocyten der Neugeborenen infolge einer sehr niedrigen Konzentration von Phosphoglycerinsäure einen Gehalt an organischem, säurelöslichem Phosphat, der unter demjenigen des ausgewachsenen Tieres liegt.

Wir stellen weiterhin fest, dass die bedeutenden Speziesunterschiede der Adulttiere in frühen Ontogenesephasen kaum hervortreten.

The Anti-Tumour Activity of 6-Azaauracil Riboside

It has recently been found in this Laboratory¹, and also independently by HANDSCHUMACHER and WELCH², that 6-azauracil inhibits the growth of a number of microorganisms. In the case of *Escherichia coli*, the inhibitory effect has a competitive character and may be cancelled by the addition of uracil, cytosine and uridine³. 6-Azaauracil has a marked inhibitory effect on a number of experimental cancer tumours⁴. It has been observed that when *Escherichia coli* is cultivated in the presence of 6-azauracil, some 6-azauracil riboside is formed; the formation of small amounts of the riboside has also been detected with *Streptococcus fecalis*⁵. Preliminary experiments have revealed pronounced cancerostatic effects of 6-azauracil riboside⁶; a detailed examination has not hitherto been possible due to lack of material.

More recently we have devised a method for the production of 6-azauracil riboside by a fermentation procedure which has made possible experiments *in vivo*⁷.

We first examined the effect of 6-azauracil riboside on Ehrlich's ascites tumours in subcutaneous form in white mice (strain H). Mice (weight 20 g) were subcutaneously inoculated with 0.2 ml of a diluted 8 days old ascites Ehrlich tumour (about 13×10^6 cells). Chemotherapy was

started on the fifth day following transplantation. The riboside was applied in physiological solution in 0.2 ml lots subcutaneously as far as possible from the tumour inocula. The daily dose of the drug given for 7 days amounted to 500, 250 and 50 mg pro 1 kg of weight of the test animal. 24 h after the last dose the tumours were taken out and weighed.

The results are given in the Table.

	Weight of tumor	P	Slowing down of growth of experimental tumour in %
Controls	348 ± 40.3		
500 mg azauracil riboside/kg	96.3 ± 11.5	< 0.01	72
Controls	559 ± 68.5		
250 mg azauracil riboside/kg	194.5 ± 21	< 0.01	65
50 mg azauracil riboside/kg	365.5 ± 52	0.037	35
* Controls	1169 ± 102		
500 mg azauracil/kg.	890 ± 73	0.03	24

* The experiment with 6-azauracil is reported for comparison.

Our experiments show that in the 500 mg/kg dose 6-azauracil riboside is about 3 times more effective than 6-azauracil administered in the same concentration. When compared on a mole per mole basis, the riboside may be seen to be some 6 times more active than 6-azauracil itself. It should be noted, on the other hand, that 6-azauracil riboside has only a relatively weak inhibitory effect on the growth of *Escherichia coli*. It may be assumed that the mechanism of action of the two antimetabolites against tumour and microbial cells differs somewhat in character.

Dr. HÁVA and Mr. JANKŮ in the Pharmacological Laboratory of this Institute have found that the toxicity of 6-azauracil riboside, like that of 6-azauracil, is very low: a daily dose of 500 mg/kg, administered intraperitoneally for 8 days to white mice (strain H), had no adverse effects.

Clinical experiments with 6-azauracil riboside are now in progress.

F. ŠORM and H. KEILOVÁ

Institute of Chemistry, Czechoslovak Academy of Science, Prague, February 12, 1958.

Zusammenfassung

Es wird die cancerostatische Wirkung des 6-Azaauracil-ribosids auf die subkutane Form des Ehrlich-Aszites-Tumors beschrieben und festgestellt, dass 6-Azaauracil-ribosid ungefähr 6mal wirksamer ist als 6-Azaauracil.

Die Hemmbarkeit der inhibitorischen Adrenalin- bzw. Noradrenalinwirkung durch Adrenolytika

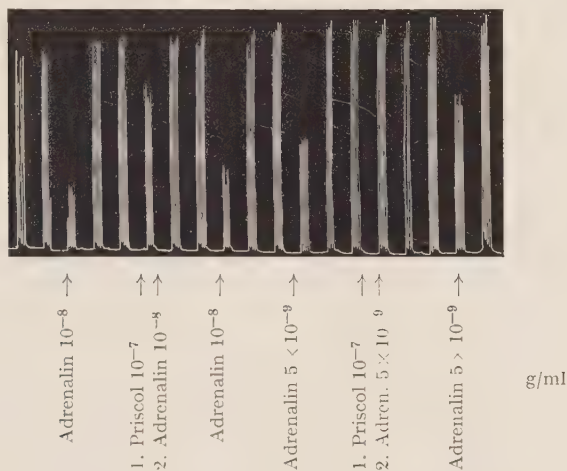
Im Gegensatz zu den exzitatorischen Effekten des Adrenalins gelang die antagonistische Beeinflussung inhibitorischer Effekte durch Adrenolytika überzeugend nur am spontan arbeitenden Kaninchendarm. Für eine Gruppe dieser Substanzen, die β -Haloalkylamine, war der Nachweis eines Antagonismus bisher überhaupt nicht möglich.

¹ F. ŠORM and J. ŠKODA, Chem. listy 50, 827 (1956); Coll. Czechosl. chem. Comm. 21, 487 (1956).
² R. E. HANDSCHUMACHER and A. D. WELCH, Cancer Res. 16, 965 (1956).
³ J. ŠKODA and F. ŠORM, Chem. listy 50, 1165 (1956); Coll. Czechosl. chem. Comm. 21, 1328 (1956).
⁴ F. ŠORM, A. JAKUBOVIČ, and L. SLECHTA, Exper. 12, 271 (1956). T. M. HAKALA, L. W. LAW, and A. D. WELCH, Proc. Amer. Ass. Cancer Res. 2, 113 (1956). – J. ŠABLÍK and F. ŠORM, Neoplasma 4, 113 (1957). – H. KEILOVÁ and F. ŠORM, Neoplasma 4, 204 (1957).
⁵ J. ŠKODA, V. F. HESS, and F. ŠORM, Exper. 13, 150 (1957); Chem. listy 51, 1195 (1957); Coll. Czechosl. chem. Comm. 22, 1330 (1957).
⁶ J. ŠKODA and F. ŠORM, Chem. listy 50, 1165 (1956); Coll. Czechosl. chem. Comm. 21, 1328 (1956). – J. ŠKODA, V. F. HESS, and F. ŠORM, Exper. 13, 150 (1957); Chem. listy 51, 1195 (1957); Coll. Czechosl. chem. Comm. 22, 1330 (1957). – R. E. HANDSCHUMACHER, Biochim. biophys. Acta 23, 428 (1957). – R. SCHINDLER and A. D. WELCH, Proc. Amer. Ass. Cancer Res. 2 (3), 247 (1957).
⁷ J. ŠKODA and F. ŠORM, Biochim. biophys. Acta (in press).

In unseren Untersuchungen wurden N,N-Dibenzyl- β -chloräthylamin (Dibenamin), Dihydroergotamin, Phentolamin (Regitin) und Tolazolin (Priscol) am submaximal elektrisch gereizten Ileum des Meerschweinchens gegen Adrenalin ausgewertet (Methode s. KUSCHINSKY, HÄRTFELDER und MOSLER¹). Durch Adrenalin bzw. Noradrenalin liessen sich die durch elektrische Reizung ausgelösten Kontraktionen in Abhängigkeit von der Dosis um 10 bis 100% hemmen. Die notwendigen AdrenalinKonzentrationen betrugen 10^{-9} – 10^{-7} g/ml.

Bei geeigneter Dosierung liess sich mit Priscol eine 40–90%ige Adrenalin- oder Noradrenalinhemmung abschwächen oder aufheben (s. Abb. 1). Priscol hatte in der zur Darstellung des Adrenalinantagonismus notwendigen Dosis keine Eigenwirkung. Eine etwa gleich starke durch Papaverin hervorgerufene Depression wurde durch Priscol nicht beeinflusst. (Dies gilt auch für die anderen untersuchten Sympatholytika.)

Durch Regitinvorgabe liess sich die Adrenalinhemmung völlig aufheben, doch war der Ausgangszustand auch nach mehrmaligem Auswaschen nicht mehr ganz zu erreichen. Bei einem Dosenverhältnis von 1:1 und einer Endkonzentration von 10^{-8} g/ml war der Antagonismus zu Adrenalin optimal.



eneur en acide citrique ($\mu\text{g/g}$ de tissu frais) des organes de la souris après injection de fluoroacétate (FA) et irradiation éventuelle à la 5^e h (X).

	Souris témoins	5 h après FA	14 h après FA	14 h après FA, 9 h après X	9 h après X seuls
Reins	23	1333	1155	552	0
Myocarde	12	617	864	882	0
Rate	19	585	631	354	54 (?)
Encéphale	20	111	88	62	12
Foie souris ♂	94	157	150	70	50
souris ♀	66	84	81	77	60

Souris C 57 noires de 18 à 22 g, nourries *ad libitum*. L'acide citrique est dosé par la méthode de TAYLOR². Les valeurs de ce tableau sont des moyennes obtenues sur au moins 6 animaux. Les variations du taux d'acide citrique dans le foie des souris ♀ ne sont pas statistiquement significatives. L'effet antagoniste des rayons X (775 r, Stabilivolt Siemens 200 kV) sur l'accumulation d'acide citrique, produite dans le myocarde par injection de fluoroacétate aux animaux, n'apparaît qu'après la 14^e h. La dose de fluoroacétate sodique utilisée (5 mg/kg) tue environ 30% des animaux dans les 3 jours.

et STOCKEN³ ont constaté en effet que le foie des rats nourris accumule plus de citrate, après injection de fluoroacétate, que celui des rats maintenus à jeun 24 h avant l'injection du toxique. BUSCH *et al.*⁴ ont montré d'autre part que des coupes de foie incubées en présence de fluoroacétate accumulent du citrate si elles disposent de substrats oxydables appropriés et si la pression partielle d'oxygène dans les coupes est suffisante.

L'influence des hormones sexuelles semble également évidente a) puisque les valeurs de citrate accumulé sont plus élevées dans le foie des rattes que dans le foie des rats³, b) puisque la castration des rats mâles augmente leur capacité d'accumuler du citrate dans leur foie⁵, tandis que la même opération sur les rattes produit plutôt l'inverse⁶, c) puisque enfin l'accumulation de citrate dans le foie des rattes intoxiquées ne se manifeste qu'à l'âge adulte⁶.

L'irradiation totale des rats diminue leur capacité d'accumuler du citrate dans leurs tissus sous l'influence d'une injection ultérieure de fluoroacétate⁷; cependant le foie des rats mâles accumule dans ce cas beaucoup plus de citrate⁷. Certains des effets immédiats et plus tardifs des irradiations pourraient être dus à une action des rayons X sur les hormones androgènes⁷.

Au cours de recherches entreprises en vue d'interpréter la radio-protection qu'assure à la souris l'injection préalable de doses sub-léthales de fluoroacétate⁸, nous avons constaté que le foie des souris se comporte à l'opposé du foie des rats. Le foie des souris mâles accumule du citrate après administration de fluoroacétate; le foie des souris femelles ne le fait pas. Nous n'avons pas recherché l'effet

du fluoroacétate à divers âges. Il est peu probable cependant que l'absence d'accumulation de citrate dans le foie des souris femelles soit dû au trop jeune âge des animaux; ces souris pesaient 18 à 22 g et devaient avoir plus de 12, sinon plus de 14 semaines.

L'irradiation des souris mâles 5 h après l'injection du toxique fait tomber leur taux de citrate hépatique à la moitié du taux du citrate hépatique de souris témoins intoxiquées mais non irradiées. Le foie des souris mâles répond par conséquent aux irradiations comme les autres tissus de cet animal⁹. Le foie du rat mâle par contre, accumule du citrate après empoisonnement au fluoroacétate si l'animal a été préalablement irradié⁷. La différence de comportement de ces deux espèces animales ne peut être due à l'âge de nos souris mâles: à 18–22 g, les souris mâles ont plus de 10 semaines et sont adultes. Remarquons que nous avons irradié les souris quelques heures après administration du poison alors que dans les expériences faites chez le rat⁷, l'irradiation précède l'administration de fluoroacétate.

Nous tenons à remercier le Dr. A. HERVE, chef de travaux à l'Institut de Radiologie, qui a irradié les animaux.

C. LIÉBECQ et SUZANNE LIÉBECQ-HUTTER

Laboratoires de Biochimie de l'Université de Liège et Laboratoires de Recherches pour la Protection des Populations civiles, Liège (Belgique), le 17 mars 1958.

Summary

In contrast to rats, male mice injected with sodium fluoroacetate accumulate citrate in their livers, whereas females do not. Whole-body irradiation reduces the level of accumulated citrate in the liver as well as in other tissues of the mouse.

⁹ C. LIÉBECQ et S. LIÉBECQ-HUTTER, Arch. int. Physiol. 66, 77 (1958).

The Oral Route for the Bio-Assay of Aldosterone-like Materials in the Rat: Relative Potency of Aldosterone and Chloro-Cortisol

Although numerous methods have been described (for reviews see ¹) for the bio-assay of aldosterone-like materials, no method for quantitative assay has been hitherto reported in which the steroids were administered by mouth. It seemed of interest to investigate the feasibility² of this route because (a) substances like liquorice and glycyrrhetic acid have been reported³ as having aldosterone-like activity in humans; and (b) the discovery⁴ of new steroids capable of blocking the effects of aldosterone and DCA on electrolyte excretion makes it desirable to assess the administration by oral route in order to widen their possible therapeutic usefulness. It seems reasonable to postulate that any attempt to assay in the rat the oral effectiveness of an aldosterone antagonist on Na⁺ and K⁺, must be based on a satisfactory dose-

² T. G. TAYLOR, Biochem. J. 54, 48 (1953).

³ M. G. ORD et L. A. STOCKEN, Proc. Soc. exp. Biol. Med. 83, 395 (1953).

⁴ H. BUSCH, J. R. DAVIES et E. W. OLLE, Fed. Proc. 16, 161 (1957).

⁵ M. G. ORD et L. A. STOCKEN, Proc. Soc. exp. Biol. Med., 83, 395 (1953). – K. P. DuBOIS, K. W. COCHRAN et M. M. ZERWIC, Proc. Soc. exp. Biol. Med. 78, 452 (1951).

⁶ K. P. DuBOIS, K. W. COCHRAN et M. M. ZERWIC, Proc. Soc. exp. Biol. Med. 78, 452 (1951).

⁷ K. P. DuBOIS, K. W. COCHRAN et J. DOULL, Proc. Soc. exp. Biol. Med. 76, 422 (1951).

⁸ Z. M. BACQ, P. FISCHER et A. HERVE, Arch. int. Physiol. 66, 75 (1958).

¹ R. I. DORFMAN, Physiol. Rev. 34, 138 (1954). – J. G. LLAURADO, Klin. Wschr. 34, 669 (1956).

² J. G. LLAURADO, Proc. Univ. Otago Med. Sch. 35, 23 (1957).

³ J. G. G. BORST, S. P. TEN HOLST, L. A. DE VRIES, and J. A. MOLHUYSEN, Lancet 1953, I, 657.

⁴ C. M. KAGAWA, J. A. CELLA, and C. G. VAN ARMAN, Science 126, 1015 (1957).

Comparison of aldosterone-like effect between bio-assays by oral and subcutaneous route

Steroid	Oral				Subcutaneous			
Dose in μg	N^1	k^2	Response mean $\pm$ S. E. ³	$P <^4$	N	k	Response mean $\pm$ S. E.	$P <$
Aldosterone								
0.015	—	—	—	—	14	2	86.91 $\pm$ 7.47	0.2
0.05	—	—	—	—	14	2	59.86 $\pm$ 5.00	0.001
0.06	13	2	88.99 $\pm$ 7.40	0.3	—	—	—	—
0.15	—	—	—	—	14	2	33.55 $\pm$ 3.53	0.001
0.20	14	2	83.03 $\pm$ 7.67	0.05	—	—	—	—
0.50	—	—	—	—	14	2	22.27 $\pm$ 2.72	0.001
0.60	14	2	48.62 $\pm$ 5.08	0.001	—	—	—	—
2.00	14	2	29.11 $\pm$ 3.51	0.001	—	—	—	—
0.68	—	—	50	—	—	—	—	—
0.089	—	—	—	—	—	—	50	—
Regression line ⁵	$y + 42.27x = 42.99$				$y + 43.63x = 4.29$			
S. D. L. ⁶	8.90				6.28			
S. E. ⁷ _p	6.99				4.93			
$L = b /S. D. L.^8$	4.75				6.95			
r^9	— 0.97				— 0.98			
Cl-F								
0.05	7	1	90.91 $\pm$ 19.05	0.7	7	1	79.47 $\pm$ 15.17	0.2
0.10	14	2	80.77 $\pm$ 14.37	0.2	7	1	68.26 $\pm$ 7.93	0.01
0.25	13	2	72.00 $\pm$ 9.34	0.01	6	1	37.89 $\pm$ 4.90	0.001
0.50	20	3	53.75 $\pm$ 3.59	0.001	5	1	35.58 $\pm$ 5.55	0.001
1.00	14	2	26.47 $\pm$ 0.92	0.001	—	—	—	—
5.00	6	1	13.25 $\pm$ 1.39	0.001	—	—	—	—
10.00	6	1	9.27 $\pm$ 1.70	0.001	—	—	—	—
0.59	—	—	50	—	—	—	—	—
0.20	—	—	—	—	—	—	50	—
Regression line	$y + 38.40x = 41.21$				$y + 48.59x = 16.38$			
S. D. L.	8.05				6.77			
S. E. _p	3.88				8.90			
$L = b /S. D. L.$	4.77				7.19			
r	— 0.97				— 0.97			

¹ Total number of rats in dose assayed group. An equal number of rats was used as control for each group.

² Number of bio-assays.

³ Standard error calculated as given by WORTHING and GEFNER¹⁰.

⁴ Significance of difference between assay and control groups.

⁵ $x = \log X$; $X = \text{dose in } \mu\text{g}$.

⁶ Standard deviation about the line.

⁷ Standard error of slope.

⁸ Estimate of index of precision.

⁹ Coefficient of correlation.

¹⁰ A. G. WORTHING and J. GEFNER, *Treatment of Experimental Data* (Wiley, New York 1943), p. 207.

response relationship previously obtained by using aldosterone-like corticoids *per os* in the same type of bio-assay.

It was shown earlier⁵ that aldosterone and 9 α -chloro-cortisol acetate (Cl-F) are very potent substances in promoting a fall of the urinary Na⁺/K⁺ ratio, the latter taken as an index of electrolyte-regulating activity in the rat. For this reason these corticoids were chosen for the present investigation. The method of bio-assay by subcutaneous route in the young adrenalectomized male white rat has been previously⁶ described. For the oral route the same method was used, the only difference being that each steroid was administered by an intra-gastric tube in a volume of 0.15 ml (instead of 0.1 ml) of

20% ethanol/water as solvent. The activity of aldosterone and Cl-F was indicated by a fall in the Na⁺/K⁺ ratio in the urine of the rats, this ratio being expressed as a percentage of the mean for a control group. Thus a more intense effect is represented by a greater reduction of this percentage. Aldosterone was administered as D,L-aldosterone 21-mono-acetate. The *biological* activity of this chemical form is 4 tenths that of the non-acetylated D-aldosterone⁷. The doses of aldosterone listed in the Table express quantities of D-aldosterone, the amounts of the racemic aldosterone acetate actually given to each rat being therefore 2.5 times greater.

The results together with several calculated statistical parameters are shown in the Table. Values obtained by the

⁵ J. G. LLAURADO, *Exper. 11*, 401 (1955); *Klin. Wschr.* 34, 674 (1956).

⁶ J. G. LLAURADO, *Endocrinology* 58, 390 (1956); *Klin. Wschr.* 34, 669 (1956).

⁷ S. A. SIMPSON, J. F. TAIT, A. WETTSTEIN, R. NEHER, J. v. EUW, O. SCHINDLER, and T. REICHSTEIN, *Helv. chim. Acta* 37, 1163 (1954); Notice in *J. clin. Endocrinol.* 17, 699 (1957).

subcutaneous route are also given for comparison. It was found that there is a linear relationship between the fall of the Na^+/K^+ ratio and the logarithm of the dose of each corticoid administered by mouth in the following ranges: aldosterone 0.06–2.00 μg ; Cl–F 0.05–10.00 μg . As expected the response obtained by the oral route for each comparable dose level was less than that obtained when the same steroid was given subcutaneously. From the regression lines tabulated in the Table, the dose of each steroid which produces a 50% effect on the Na^+/K^+ ratio has been calculated, this being for the oral route 0.68 μg of aldosterone and 0.59 μg of Cl–F, and for the subcutaneous route 0.089 μg and 0.20 μg respectively.

Aldosterone, when administered by subcutaneous route, is at least twice as potent as Cl–F, as judged by comparison of the calculated dose of each steroid which produces a 50% response. In this context it is of great interest that aldosterone is *less* effective than Cl–F when given by mouth (Table). These observations reinforce the hypothesis⁸ that the increased activity of some halogenated steroids as compared with 'natural' hormones may result from a slower rate of metabolism by the liver.

The conclusions to be drawn from this experiment are: (a) the oral route, though not as sensitive as the subcutaneous, can be used for the bio-assay of aldosterone-like materials in the rat; and by inference, for the assessment of aldosterone antagonists; (b) aldosterone is less effective by mouth than Cl–F; (c) the relative potency on electrolyte-regulating activity of two or more different steroids tested by the subcutaneous route should not be used as an indication of their relative potency by the oral route.

We wish to thank Mr. G. F. S. SPEARS of Otago University Medical School, for much assistance in the statistical analysis, and the Squibb Institute for Medical Research, New Brunswick, New Jersey, for kindly supplying the halogenated steroid. Aldosterone was generously given by Dr. R. GAUNT from CIBA, Summit, New Jersey at the request of the Endocrinology Study Section, National Institutes of Health, Bethesda 14, Maryland.

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Résumé

On démontre que chez le rat, l'administration orale de l'aldostérone et substances similaires pour essai biologique est praticable et sûre, bien que moins sensible que l'administration parentérale. Par inférence, il est suggéré que cette méthode est utilisable pour l'évaluation des antagonistes de l'aldostérone.

A l'inverse de leur efficacité relative par voie parentérale, l'aldostérone est par voie orale moins actif chez le rat que l'acétate de 9 α -chlorocortisol pour produire l'abaissement du rapport Na^+/K^+ .

⁸ E. M. GLENN, R. O. STAFFORD, S. C. LYSTER, and B. J. BOWMAN, *Endocrinology* 61, 128 (1957).

The Effect of 17 α -Ethyl-19-Nortestosterone on the Estrus Cycle

17 α -Ethyl-19-nortestosterone (Nilevar®)¹ is reported to show a potent anabolic², progestational³, and deciduomagenic⁴ activity in rats, an anti-estrogenic effect in spayed estrogen-treated rats⁵, to inhibit the pituitary gland in parabiotic rats⁶, and to damage the reproductive process as evidenced by the inhibition of the ovulation in rabbits⁴ and by the antifertility⁴ effect in rats. In the course of our investigations on the possibly protective effects of this new synthetic hormone in adult female, ethionine-treated intact rats, we have observed that the estrus cycle was completely inhibited during the time of the intramuscular administration of 0.5 mg/kg body weight, a dose being highly anabolic, but not yet androgenic⁷. Since the animals had to be killed after 13, or 22 days respectively, it cannot be said whether or when the normal cycle would have been restored after the medication has been stopped. Testosterone propionate in corresponding non-adrogenic doses⁸, i.e. 0.04 mg/kg body weight, did not inhibit the estrus cycle: Only 2 out of 16 animals had a prolonged anestrus for the first 12, or 13 days respectively. All control animals treated with the vehicle (sesame oil) alone, showed no change of their normal cycles. The estrus cycle-interrupting effect of 17 α -ethyl-19-nortestosterone can be explained by the known inhibition of the pituitary gland⁶ and/or by the anti-metabolite effect on the estrogens⁵ present in the intact animal. Further detailed studies are in progress.

R. RICHTER

Universitätsfrauenklinik Bern, 12. März 1958.

Zusammenfassung

Im Verlaufe unserer Untersuchungen mit 17 α -Äthyl-19-nortestosteron (Nilevar) beobachteten wir, dass bei erwachsenen weiblichen, mit diesem neuen Hormon behandelten Ratten kein Östrus mehr auftrat, während Testosteronpropionat in entsprechenden, nicht androgenen Dosen den östrischen Zyklus nicht signifikant beeinflusste.

¹ Appreciation is expressed to Dr. V. A. DRILL, of G. D. Searle & Co., Chicago, Ill., USA., for generous supplies of Nilevar®.

² R. R. McSWINEY and F. T. G. PRUNTY, *J. Endocrinol.* 13, xxv (1956). – F. J. SAUNDERS and V. A. DRILL, *Endocrinology* 58, 567 (1956). – R. C. KORY, R. W. WATSON, M. H. BRADLEY, and B. J. PETERS, *J. clin. Invest.* 36, 907 (1957). – F. J. SAUNDERS and V. A. DRILL, *Proc. Soc. exp. Biol. Med.* 94, 646 (1957).

³ F. J. SAUNDERS, F. B. COLTON, and V. A. DRILL, *Proc. Soc. exp. Biol. Med.* 94, 717 (1957).

⁴ G. PINCUS, M. C. CHANG, M. X. ZARROW, E. S. E. HAFEZ, and A. MERRILL, *Endocrinology* 59, 695 (1956).

⁵ R. A. EDGREN, *Acta endocr.* 25, 365 (1957).

⁶ J. N. GOLDMAN, J. A. EPSTEIN, and H. S. KUPPERMAN, *Endocrinology* 61, 166 (1957).

⁷ F. J. SAUNDERS and V. A. DRILL, *Proc. Soc. exp. Biol. Med.* 94, 646 (1957). – R. E. RANNEY and V. A. DRILL, *Endocrinology* 61, 476 (1957).

⁸ R. E. RANNEY and V. A. DRILL, *Endocrinology* 61, 476 (1957).

Electrical Responses of Astrocytic Glia from the Mammalian Central Nervous System Cultivated *in vitro*¹

During the past years methods for the cultivation of various areas of the mammalian central nervous system have been developed². In addition to neurons these explants provide large quantities of glial cells which become, in the course of cultivation, more or less isolated so that they can easily be observed with phase contrast microscopy. Time lapse cinematographic records revealed that such astrocytes can exhibit slow rhythmical movements³ similar to those of amoebae or slime molds. For the present study, astrocytes from the midbrain and from the cerebellum of the kitten were used which were cultivated according to our standard methods on 12 × 50 mm cover slips in roller tubes. The cultures were kept in the incubator at 37°C for 30 to 50 days before they were used for electrophysiological experiments. By this time the astrocytes were separated from one another to such a degree that single elements could be easily distinguished (Fig. 1).

The cultures were mounted in a chamber approximately 4 mm deep. The bottom of this chamber consisted of a microscopic slide cut to approximately 12 mm in width and its roof was formed by the cover slip on which the cultures were grown. After the chamber was filled with Gey's balanced salt solution the cells were observed with Zeiss phase contrast optics at a magnification of approximately 600×, while the recording and stimulating electrodes were introduced from below through the open sides of the chamber at an angle of 10 to 30 degrees with the aid of a modified Leitz micromanipulator. The placement of the electrodes in and near the cells was made under visual control. The recording microelectrodes were filled with 3 M KCl-solution and had a tip diameter of less than 0.5 μ and a direct current resistance between 10 and 25 MΩ. The stimulating electrodes consisted of a pair of glass capillaries filled with Gey's balanced salt solution. The one close to the cell had a tip diameter between 5 and 15 μ, whereas the other one which was immersed some distance away in the chamber fluid was a plain glass tubing of about 1 mm in diameter.

Stimulating pulses were obtained from a Grass stimulator used in conjunction with an isolation unit. The pulse duration was approximately 1 ms. Intracellular potentials were recorded with a unity-gain preamplifier designed and constructed by BAK⁴. The input capacity of this amplifier was of the order of 0.3 pF and the grid current could be adjusted to zero at or near the working input voltage. A DuMont oscillograph was used for recording and the records were photographed with a Grass camera. In the attempt to pierce single astrocytes with the recording microelectrodes in many cases considerable difficulty was encountered due to the plasma clot in which the cells were embedded. The plasma clot made it difficult to achieve clean penetration of the cell body which, in such cases, was injured to such a degree that precise records

could not be obtained. Similar difficulties were reported by CRAIN⁵ in his experiments with chicken embryo spinal ganglia *in vitro*. Only in areas where clot liquefaction had taken place could clean penetrations of glia cell bodies be performed.

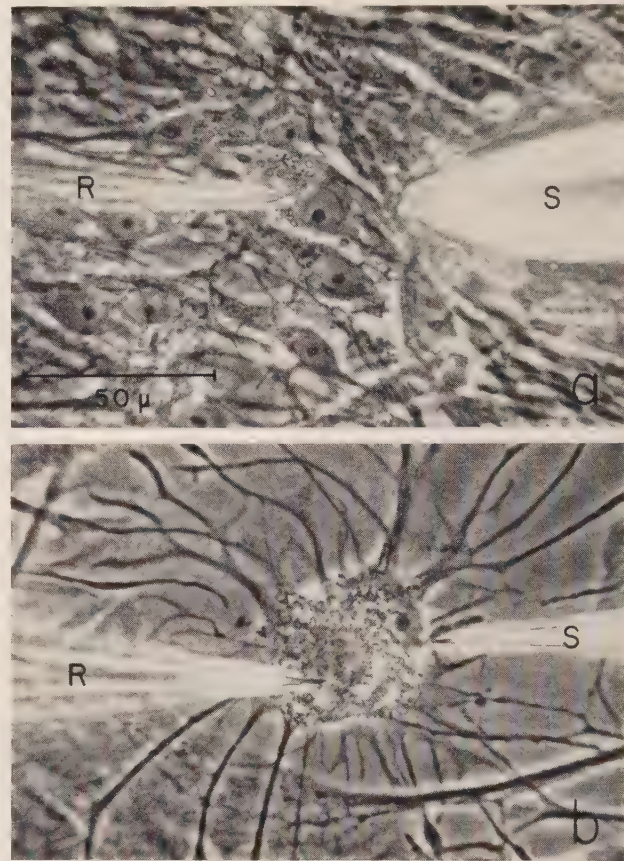


Fig. 1a and b. Photomicrographs showing representative astrocytes in the living state and the position of the electrodes during the recording time. a Field close to the center of the culture where the cells have retained their original netlike connections. A recording microelectrode (R) is introduced in one astrocyte whereas a larger stimulating electrode (S) is located near the cell. At times the stimulating electrode was used as an injection pipette by means of which a limited amount of isotonic KCl solution could be placed in the immediate vicinity of the cell. b A flatly spread out astrocyte somewhat isolated in the outer zone of outgrowth. The recording electrode (R) is introduced into the cell whereas a small stimulating electrode (S) is placed very close to the surface membrane of the cell. The outline of the nuclear membrane lies outside the plane of focus. Thirty-five day old cultures, phase contrast. The magnification is indicated by a bar in a.

On penetration of an astrocyte there was a sudden shift in the recorded direct current potential of usually less than 50 mV, the recording tip being negative to the potential of the surrounding fluid medium. On few occasions the potential shift was as large as 70 mV. When a small amount of an isotonic KCl solution was introduced by means of a small injection pipette placed in the immediate vicinity of the cell under investigation, the negativity of the intracellular potential decreased rapidly. On cessation of the outflow of the KCl solution from the injection pipette the negativity returned toward the original

¹ This investigation was supported in part by a research grant (PHS B-364[C4]) from the National Institute of Neurological Diseases and Blindness, of the National Institutes of Health, Public Health Service, administered by C. M. POMERAT.

² W. HILD, Z. Zellforsch. 40, 257 (1954). – C. M. POMERAT and I. Costero, Amer. J. Anat. 99, 211 (1956). – W. HILD, Z. Zellforsch. 46, 259 (1957); 47, 127 (1957). – M. OKAMOTO, Z. Zellforsch. 47, 269 (1958).

³ W. HILD, Z. Zellforsch. 40, 257 (1954).

⁴ A. BAK, J. EEG clin. Neurophysiol., in press (1958).

⁵ S. M. CRAIN, J. comp. Neurol. 104, 285 (1956).

level (Fig. 2, top). This observation indicates that the intracellular direct current potential of the astrocytes is similar to the resting potential of nerve or muscle fibers.

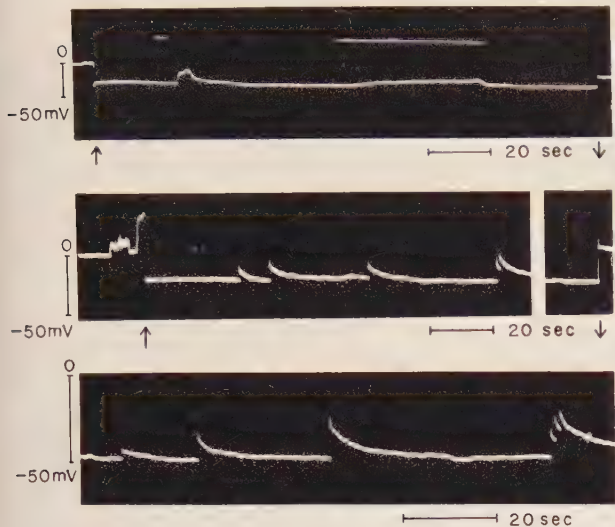


Fig. 2.—Electrical changes recorded from cultured glial cells on a continuously moving film. 0 is the potential level of the recording electrode when it is in the external fluid medium. The moment of impalement of the recording electrode into the cell is indicated by $\uparrow$, while that of removal from the cell by $\downarrow$. (Top) Shift of resting direct current level caused by the introduction of KCl solution into the immediate vicinity of the cell. Horizontal bars represent the KCl inflow; the first one was rather fast and the second quite slow. (Middle) Responses of glial cells to electrical stimuli. The shock intensities across the stimulating electrode with a resistance of $2\text{ M}\Omega$ were (from left to right): -50 V (showing almost no response), -80 V , -100 V , $+100\text{ V}$, and $+$ and -100 V at 1 s intervals. (Bottom) Similar responses to the shocks of: $+40\text{ V}$, -40 V , -50 V , and three -40 V at 1 s intervals. Note the difference in response magnitude to the cathodal and the anodal shocks of the same intensity and the summated responses to the rapidly repeated stimuli.

Application of electrical stimuli to impaled glial cells resulted in a transient reduction of their resting potential (Fig. 2, middle and bottom). No clear glial response was observed when the resting potential was less than $15\text{--}20\text{ mV}$. The degree of this potential reduction increased with the strength of the applied electric shock. The time course of the potential change was roughly exponential, the $1/e$ decay time being approximately 4 s at about 27°C . The time course was about one thousand times as long as that of the electrical response of neurons in the same culture. Unless the stimulus strength exceeded an injurious high level, the time course of the potential change was independent of the stimulus strength. With a relatively small stimulating electrode placed close to the surface of the astrocytes (Fig. 1b), a cathodal stimulus was more effective than an anodal stimulus of the same strength. When a stimulating cathode with a tip opening of about $10\text{ }\mu$ in diameter was placed at a distance of about $15\text{ }\mu$ from the surface of the cell (Fig. 1a), a distinct effect of stimulation could be observed at current intensities greater than $10\text{--}20\text{ }\mu\text{A}$.

The effectiveness of stimulating pulses applied to an astrocyte was found to increase gradually when the stimulus duration was increased from 1 m/s to 20 m/s . For stimulus durations shorter than about 0.2 m/s , the effectiveness decreased rapidly with shorter durations. A second or more stimuli following the first one after a short interval evoked a response superimposed on the falling

phase of the preceeding response (Fig. 2, middle and bottom). All these properties of the potential variation in the surface membrane of the glial cell resembled the 'electric response' of the slime mold, *Physarum polycephalum*, which was investigated by TASAKI and KAMIYA⁶.

There was no significant difference in the responses of various modulatory types of astrocytes as they are known to exist in tissue culture material. Almost completely isolated cell forms, which had flattened out to a considerable degree (Fig. 1b), showed the same responses as astrocytic elements which had remained in contact with each other as in the more central parts of the culture (Fig. 1a).

The resting potential of many cells remained uninfluenced after completely replacing the surrounding fluid medium with a cocaine ($0.05\text{--}0.1\%$) or ethyl-urethane ($1.5\text{--}2\%$) salt solution. On several occasions a strong but reversible suppression of the 'response' of the glia cells was demonstrated with these narcotics. Our present interpretation of the 'glia response' is the following: The surface membrane of the astrocyte is capable of developing an electrical response by a mechanism similar to that of the nerve or muscle fiber membrane. The interaction, however, between the 'responding' and the resting area of the membrane is not strong enough to cause re-stimulation of the resting area by the electric current arising from the responding area. An increase in the stimulus strength increases the amplitude of the observed response by decreasing the membrane area remaining unexcited by the stimulus. The low resistivity of the surface membrane appears to be the cause for the impossibility of re-stimulation.

Since astrocytes constitute a very large percentage of the entire cell population in the mammalian central nervous system, the finding that these cells can develop extremely slow electric responses may be of significance in the interpretation of slow time scale recordings taken from the intact surface of the brain. It appears important, therefore, to examine the relationship between the slow electric phenomena in the cortex, such as spreading depression, and the response of the glial cells.

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Zusammenfassung

Einzelne Astrozyten in Gewebekulturen vom Klein- und Mittelhirn junger Katzen wurden elektrophysiologisch untersucht. Ableitungen mit Mikroelektroden, die unter mikroskopischer Kontrolle in die Gliazellen eingestochen waren, zeigten Ruhepotentiale ähnlich den aus Nerven- und Muskelfasern bekannten. Durch isolierte Umspülung der Gliazellen mit isotonischer KCl-Lösung konnten diese Ruhepotentiale aufgehoben werden. Elektrische Reizung der Gliazellen verursachte eine temporäre Reduktion des Ruhepotentials für $4\text{--}5\text{ s}$. Es werden Angaben gemacht über die Reaktion der Gliazellen nach Kokainisierung und nach Urethannarkose.

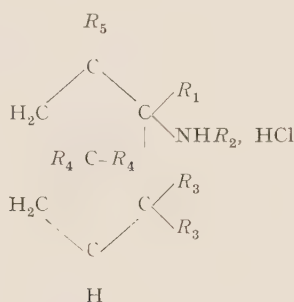
⁶ I. TASAKI and N. KAMIYA, *Protoplasma* 39, 333 (1950).
* Visiting in Galveston.

Hypotensive and Ganglion Blocking Action of Methyl Substituted 3-Amino-norcamphanes

The announcement¹ that 3-methylamino-isocamphane (Mecamylamine) (I) possessed potent ganglion-blocking properties seemed to call for a rather thorough revision of generally accepted concepts of the structure-activity relation of acetylcholine antagonism in autonomic transmission.

A study was undertaken with the object of establishing the structural requirements for appearance of ganglion-blocking action, similar in nature and potency to that exhibited by I, in amines related to this substance.

The results obtained with I and five methyl substituted 3-amino-norcamphanes, congeners of I, represented by the general formula



are shown in the Table.

The results indicate that only minor variations in the number and positions of methyl groups are permissible in order to maintain ganglion-blocking and hypotensive activity. It is evident that the carbon atom at position 2 must be substituted (214/3 is inactive). When this carbon atom is substituted with two methyl groups it is of less importance, but not entirely insignificant, whether there is a C-methyl at position 3 (compare 185/13 [= I] with 214/1). It is also seen that a methyl group at position 4 is permissible (215 is active), but it may also be absent (214/1 is active). The high activity of 185/13 taken together with the paradoxical behaviour of 213 indicate the preference for grouping the substituents in a definite spatial relation to the amine function (not taking *endo-exo* isomerism into account).

The results further indicate a prominent central factor in the hypotensive action of these compounds. Evidence for this is that the blood pressure is still decreased and that the carotis occlusion reflex is absent or suppressed after the nictitating membrane contraction has again reached normal values.

The compounds with pronounced hypotensive action elicit a reversible syndrome of central nervous origin in rats fed the substance for only short periods, while for instance Asa-214/3 does not call forth this syndrome and does not reduce blood pressure. Asa-213 has a quite potent ganglion-blocking action, but has a sustained hypertensive action.

That I is not active by any simple competitive antagonism of acetylcholine has been shown for other pharmacological preparations by BENNETT, TYLER, and ZAIMIS⁴.

¹ K. H. BEYER, C. A. STONE, J. E. BAER, and E. J. ZAWOISKI, 20th Int. Physiol. Congress, Bruxelles 1956, Abstracts of Communications, p. 94. – C. A. STONE, M. L. TORCHIANA, A. NAVARRO, and K. H. BEYER, J. Pharmacol. exp. Therap. 117, 169 (1956).

Code (Asa)	R ₁	R ₂	R ₃	R ₄	R ₅	PS ₁ ganglion blocking action, % of C ₆	S ² ganglion blocking action	anti-Ace- tylcholine action ³ , % of atrop.	BP-action in anesthetized cats ⁴ , dose mm Hg max. depression (each number one experiment) duration	C.O.R. ⁵	LD ₅₀ i.p. mg/kg mice	LD ₅₀ per os mg/kg mice	Neurotoxic symptoms, rats per os
214/3	H	Me	H	H	H	10	(+)	0.02	inactive	normal	352		59 mg/kg/day for 60 days. No abnormal symptoms
214/1	H	Me	Me	H	H	300	++	0.006	0.5 mg/kg: -5, -20, -15, -40, 1 mg/kg: -20, -20, -10, -65, -30	strongly reduced	105	290	52 mg/kg/day for 14 days. Tremor, ataxia, hypermo- tility
185/13 (= I)	Me	Me	Me	H	H	450	++	0.09	1 mg/kg: -60, -30, 2 mg/kg: -35	strongly reduced	39	92	40 mg/kg/day for 21 days. Tremor and ataxia
214/6	H	H	Me	H	H	40	+	0.006	1 mg/kg: -4, 3 mg/kg: -18	reduced 50%	113		72 mg/kg/day for 60 days. Tremor, ataxia, hypermo- tility, convulsions
213	H	Me	H	Me	Me	130	+	0.002	1 mg/kg: 3 mg/kg: +10 to +30		74		
215	H	Me	Me	H	Me	135	++	0.001	1 mg/kg: -15, 3 mg/kg: -40 (-17 after 5 1/2 h) 5 mg/kg: -20, -30, -40,	strongly reduced	51	142	

¹ Parasympathetic ganglion-blocking action (for details of technique, see ²). ² Sympathetic ganglion-blocking action, quantitative expression not possible due to length of action (for details of technique, see ²). ³ Peripheral anti-acetylcholine action (guinea pig ileum) (technique of MAGNUS³). ⁴ Chloralose-urethane anesthetized cats, intravenous injection, carotid blood pressure. ⁵ Carotis-occlusion reflex (cf. ²). ⁶ J. FAKSTORP and J. G. A. PEDERSEN, Acta pharm. tox. 10, 7 (1954). ⁷ R. MAGNUS, Pflügers Arch. ges. Physiol. 102, 123 (1904).

These observations taken together with the results presented in this paper support the view that I is not merely a ganglion blocker with a uniform peroral absorption and a prolonged action due to its peculiar recirculation in the organism. (cf. BEYER *et al.*¹).

Recent evidence⁵ incidentally also points to the importance of central factors in the hypotensive action of ganglion-blocking agents representing several of the more 'orthodox' species known to possess such action.

Asa 214/1 was submitted to a brief clinical trial. 7 patients with grave fixed hypertension were treated for 8–33 days. The daily doses were from 15 to 90 mg. Asa 214/1 had a strong hypotensive effect, which, after a single dose, could last more than 12 h. The effect was practically the same after oral and subcutaneous administration. The effective dose was about 3 times as great as by treatment with I.

Parasympathetic side-effects were less pronounced than by treatment with I.

Asa 214/1, however, is unsuited for clinical use, as 5 of the treated patients in the course of 8–16 days displayed symptoms of cerebral disorder: tremor, ataxia and choreiform movements. Moreover, one patient was hallucinated and one developed a grave psychosis. All toxic symptoms disappeared in less than 30 days after discontinuation of the treatment.

Similar cerebral toxic symptoms have been described after treatment with I⁶. The cause of the symptoms is unknown.

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Med. Afd. B., Københavns Amts Sygehus, Hellerup (Denmark) March 12, 1958.

Zusammenfassung

5 Methyl-substituierte 3-Amino-norcamphane wurden im Vergleich zu 3-Methylamino-isocamphan in bezug auf Blockierung der Synapsen der autonomen Nervensysteme und blutdrucksenkenden Wirkung im narkotisierten Tier untersucht.

Die Ergebnisse deuten auf eine ganz spezifische Konstitutions- und Konfigurationsabhängigkeit der Wirkung sowie auf einen komplexen pharmakodynamischen Mechanismus, in dem die zentrale Blutdrucksenkung eine nicht unwesentliche Rolle spielt.

Erfahrungen aus kurzzeitigen klinischen Versuchen werden mitgeteilt.

⁴ G. BENNETT, C. TYLER, and E. ZAIMIS, *Lancet* 1957, II, 218.

⁵ H. E. LAPE and J. O. HOPPE, *J. Pharmacol. exp. Therap.* 116, 453 (1956). – T. B. O'DELL, C. LUNA, and M. D. NAPOLI, *J. Pharmacol. exp. Therap.* 114, 306, 317 (1956). – T. B. O'DELL and M. D. NAPOLI, *J. Pharmacol. exp. Therap.* 120, 438 (1957). – A. S. DONTAS and M. NICKERSON, *J. Pharmacol. exp. Therap.* 120, 147 (1957).

⁶ Q. B. DEMING, M. E. HODES, J. G. EDREIRA, and A. BALTHAZAR, *N. Engl. J. Med.* 256, 739 (1957). – H. M. PERRY and H. A. SCHROEDER, *J. Amer. Med. Assoc.* 164, 1455 (1957).

Survival of Bull Spermatozoa after Exposure to 150 atm N₂ for 15 Days

In the preservation of living cells through deep freezing, controllable factors are of interest. Among such factors pressure is one that can be easily and rapidly changed. LORANT, LORANT, ANGRIST, and KORPMAN¹, among others, have used high pressure as a means for storing blood at about – 12°C without freezing it. Investigations of high pressures in relation to freezing for the preservation of living cells are not to be found in the literature. MERYMAN² has studied blood preservation through deep freezing using blood protected by a moderate amount of dextrose which was sprayed through a nozzle against the surface of liquid nitrogen (– 197°C) and thus rapidly transferred into the frozen state. It has not been possible with MERYMAN's experimental conditions to achieve vitrification as investigated by LUYET³, but the results when thawing and using the blood for transfusion are good and seem promising even for routine use. Further improvement of the method is thus of interest and could eventually be achieved if spraying was undertaken in an atmosphere under high pressure. Technically this would be possible using hydrogen or helium over liquid nitrogen.

Considering the practical value of vital deep freezing and the need to modify the process in different ways for different materials, it seems interesting to investigate the effect of high gaseous pressure on some living material of relevance in vital deep freezing. One such material is the living sperm cell. Earlier investigators⁴ have demonstrated that mammalian tissues, such as skeletal muscle and heart muscle and also blood⁵, can tolerate at room temperature hydraulic pressure of at least 1000 atm for short periods and for much longer periods if the temperature is lowered or the pressure not as high. These results are important if high pressure and release of the same is tried as a means of modifying the mechanics of freezing. They make it probable that the pressure itself would not be a first hand limiting factor. For our experiments bull sperm was chosen and was studied under pressure in a gaseous environment (packed in polyethylene tubes). Little or nothing seems to have been published concerning the tolerance of living mammalian cells against gases under high pressures. Our best knowledge seems to have been collected in deep diving and in such research the pressures hardly exceed 15 atm. A good tolerance, against higher gaseous pressures also, is evidently necessary if material for deep freezing is eventually going to be investigated and handled in atmospheres with a very much higher pressure.

As a first step in the investigation of preservative freezing under pressure, the author has tested living bull sperm with respect to the tolerance against two different gases, namely N₂ and H₂. A maximum pressure of 150 atm was used. Bull sperm was chosen because the preservative freezing of such material is already in routine use in some places and also because it is easily available from the farmers' organisations for artificial insemination. (For

¹ A. LORANT, G. J. LORANT, ANGRIST, and R. KORPMAN, *J. clin. Invest.* 32, 1005 (1953).

² H. MERYMAN and E. KAFIG, *The Freezing and Thawing of Whole Blood*. Naval. Med. Res. Inst. Rept. Project NM 0000180110 (Bethesda, Md. 1955). – H. T. MERYMAN, *Science* 124, 515 (1956).

³ B. J. LUYET and P. M. GEHENIO, *Life and Death at Low Temperatures* (Biodynamica, Normandy, Mo. 1940).

⁴ V. EBBECKE and O. HASENBRING, *Pflügers Arch. ges. Physiol.* 236, 405 (1936); 236, 416 (1936).

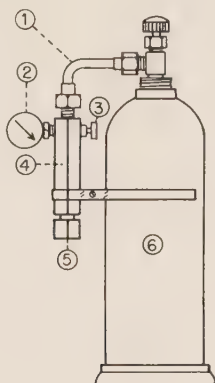
⁵ R. HAUBRICH, *Pflügers Arch. ges. Physiol.* 239, 304 (1938). – A. G. JESSIMAN and C. W. WALTER, *Surg. Forum* 36, 529 (1950).

Two experiments were made in each group

Group No.	Exposuretime	Rate of pressure change	Initial motility %	Final motility %	Comments	Initial motility %	Final motility %
Nitrogen 150 atmospheres at + 6°C						Controls at atmospheric pressure and + 6°C	
1	20 min	Slow 15 s	50-60	50-60	Only few gas bubbles No damaged spermatozoa as above (1) { Bubbles and foam. A few spermatozoa hurt with their tails lost } as above (3) as above (3)	50-60	50-60
2	20 min	Quick 2 s	60-70	60-70		60-70	60-70
3	36 h	Quick 2 s	50-60	45-55		50-60	45-55
4	7 days	Quick 2 s	70-75	45-50		70-75	35-40
5	15 days	Quick 2 s	70-75	8-10		70-75	2-3
Hydrogen 100 atmospheres at + 6°C						Controls at atmospheric pressure and + 6°C	
6	7 days	Quick 2 s	45-50	2-3	Bubbles and foam.	45-50	8-10
7	11 days	Quick 2 s	45-50	a single per vision field	A few spermatozoa hurt with their tails lost as above (6)	45-50	2-3

recent literature on vital deep freezing of sperm and living tissue see⁶.)

Sperm Preparation.—The sperm was prepared as follows. The ejaculate, which was collected at body temperature (+ 37°C), was diluted with a fluid containing 20 parts of egg yolk in 80 parts of a 2.9% Na-citrate solution with 0.1% streptomycin. This diluent was also at body temperature. The suspension was slowly cooled down to about + 4°C at a rate not exceeding 0.5°C per min. The suspension was kept cool at + 4°C for 24 h and then used experimentally after the material had been judged and estimated with respect to viability. This estimation was done under the microscope on a heated table.



1 Connecting-tube. — 2 Manometer. — 3 Needle valve to release pressure and to control the rate of pressure change. — 4 Pressure chamber. — 5 Bottom-plug. — 6 Gas-cylinder.

Pressure Apparatus.—The pressure was obtained from a 7-m³ cylinder of compressed N₂ (150 atm) or H₂ (100 atm) which could be connected to a pressure chamber

(see Figure). The chamber was made of 50 mm hexangular brass with a central bore of 10 mm. A threaded bottom-plug with high pressure packing permitted the opening and closing of the same when removing or introducing sample-tubes. A manometer to indicate the pressure and a needle valve to release the same, and also to control its rise and fall, completed the arrangement.

Sampling Technique.—The split sample method was used. Sperma suspension was drawn into a syringe in a cooling room at about + 6°C and put into thin polyethylene tubes of 4 mm internal and 4.5 mm external diameter. Each tube was 120 mm long. The tubes were first sealed at one end then filled and sealed also at the other by means of conical stoppers of ash wood. No air bubbles were left inside the tube. The stoppers were boiled for 1 h in distilled water. 8 filled tubes were used in each experiment and divided into 2 groups of 4. Of these one group served as control and was merely stored in the cooling room, whereas the other was put into the pressure chamber and exposed at cooling room temperature. The first experiments were made with N₂ at 150 atm. In later experiments H₂ at 100 atm was used because no other was available.

After each exposure 2 samples were taken out and their motility at + 37°C was compared microscopically with 2 samples from the controls. Except for this, all work was done in the cooling room at + 6°C.

In all, 7 different procedures were examined. Sperma suspension in polyethylene tubes was exposed to nitrogen at 150 atm for periods of 20 min, 36 h, 7 and 15 days. The effect of rapid (2 s) and slow (15 s) compression and decompression were also studied in the 20 min periods. During this short exposure the material was subjected to 3 serial compression-decompression cycles to test whether these repeated pressure changes would cause measurable damage.

2 experiments on the effect of hydrogen at 100 atm were made with exposure times of 7 and 11 days. In both cases pressures were applied and released in 2 s.

In every procedure the motility of the sperm was determined before and after exposure and compared with a control group at atmospheric pressure. Observations on the formation of bubbles or foam and also on mechani-

⁶ A. PARKES and A. U. SMITH, Proc. Roy. Soc. London 140, 455 (1953). — K. EIBL, B. URBASCHEK, and H. T. ZODER, Fortpflanzung, Zuchthygiene und Haustierbesamung 4, 97, 109 (1954). — K. EIBL and H. T. ZODER, Fortpflanzung, Zuchthygiene und Haustierbesamung 5, 88 (1955). — C. W. EMMENS and A. W. BLACKSHAW, Physiol. Rev. 36, 277 (1956). — C. POLGES, Proc. Roy. Soc. [B] 13, 929 (1957).

cal damage of the spermatozoa were recorded in each case. The results are summarized in the Table.

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February 20, 1958.*

Zusammenfassung

Bullenspermien überleben in N_2 bei 150 atm und $+6^\circ C$ gut 8 Tage (bis 14 Tage). In H_2 bei 100 atm und $+6^\circ C$ nimmt die Überlebensfähigkeit ab. An den Spermien wurden Schäden durch Entwicklung von Gasbläschen bei Drucksenkung beobachtet. Weitere Versuche bei höherem Druck sind in reiner Flüssigkeitsumgebung auszuführen.

Die Wirkung von 4,6-Dinitro-o-kresol auf die Atmung eines experimentellen Rattentumors bei verschiedenen Temperaturen

Da sich die Wirkung von 4,6-Dinitro-o-kresol (DNOC) auf die Gewebsatmung des Warmblüters unter gleichzeitiger Berücksichtigung des Einflusses der Temperatur wesentlich von der auf die Gewebsatmung des Kaltblüters unterscheidet¹, andererseits aber auch am Tumorgewebe eine Aktivierung der Atmung mit Dinitrophenol-Verbindungen zu erzielen ist², dürfte auch an diesem Gewebe der Temperatur eine Bedeutung zukommen. Wir untersuchten daher den Einfluss von 10^{-5} M DNOC auf die *in vitro*-Atmung eines experimentellen Rattentumors, der durch subkutane Injektion von 9,10-Dimethyl-1,2-benzanthracen erzeugt wurde und ein Spindelzell-Sarkom darstellte.

Der Temperaturbereich war $22,5-47,5^\circ C$. Atmungsmessung im indirekten Verfahren nach WARBURG mit Krebs-Bicarbonat-Ringer (ohne Glucose!) als Medium. Versuchsdauer 3 h DNOC wurde entweder zu Versuchsbeginn oder nach 1 h zugesetzt. Sonstige methodische Einzelheiten wie in vorhergehenden Untersuchungen³.

Es ergibt sich (Abb. 1), dass die Tumordatmung schon mit Temperaturerhöhung stark ansteigt, aber durch die angewandte DNOC-Konzentration noch weiter erhöht wird. Die Abbildung 2, welche die Umformung der Resultate in relative Aktivitäten bringt, lässt erkennen, dass diese Förderung systematisch mit Erhöhung der Temperatur erfolgt. Sie offenbart somit einen Verlauf, der im Gegensatz zu dem des Normalgewebes des homoiothermen Tieres, etwa der Leber der Maus¹, steht. Die %-Aktivitäts-Temperatur-Relation des Tumors entspricht daher dem Hemmtyp I nach JOHNSON⁴, das heisst einer Bindung des Inhibitors bzw. Aktivators an aktives und denaturiertes Enzym und ergibt somit eine Beziehung, welche die Atmung der Leber von Kröten unter dem kombinierten Einfluss von DNOC und Temperatur zeigte und welche als für das Gewebe des poikilothermen Tieres typische angesehen werden konnte. Im Sinne der Theorie von JOHNSON muss demnach auch für das Tumorgewebe ein Enzymgleichgewicht von der Art angenommen werden, dass sich aktives und denaturiertes Enzym konzentrationsmässig nahestehen. Dadurch unterscheidet sich das Tumorgewebe wesentlich vom normalen Gewebe des

homoiothermen Tieres, für welches eine extreme Verschiebung des Enzymgleichgewichtes zugunsten der aktiven Phase charakteristisch zu sein scheint¹. Es geht offenbar die dem Tumor zugrundeliegende Korrelationsstörung so weit, dass der Tumor sozusagen poikilotherm im homoiothermen Organismus wird.

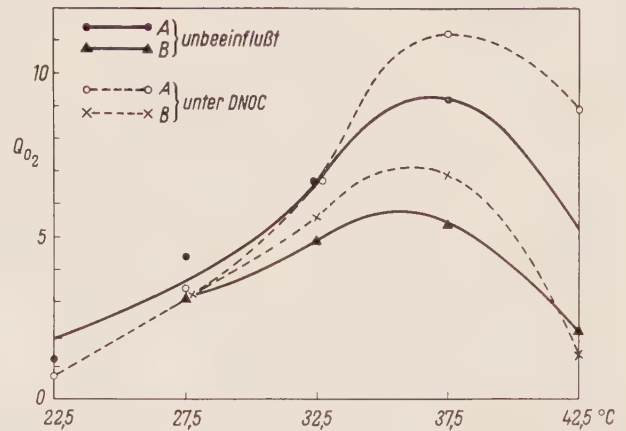


Abb. 1. Atmung des Ratten-Sarkoms unter Temperatur- und DNOC-Einfluss. A Q_{O_2} von 1.-3. h. B Q_{O_2} von 2.-3. h (bei DNOC-Zusatz 1 h nach Versuchsbeginn).

Eine weitgehende Übereinstimmung der DNOC- und Temperaturwirkung auf Kaltblüter- und Tumorgewebe besteht auch darin, dass sich beim Tumor die Aktivierungsenergie (μ) der Atmung gleichfalls erhöht: Bei DNOC von Anfang: $\mu = 18\,300 \rightarrow 27\,800$ cal, bei DNOC nach 1 h: $14\,500 \rightarrow 18\,700$ cal.

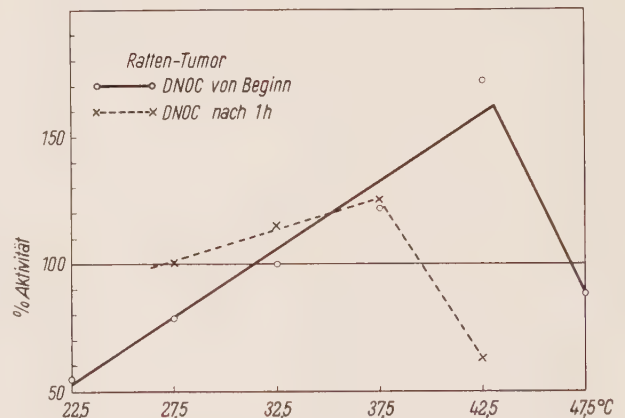


Abb. 2. %-Aktivität der Atmung des Rattentumors unter Einfluss von DNOC bei verschiedenen Temperaturen.

Für eine Übereinstimmung der morphologischen Organisation des Tumorgewebes des Warmblüters mit Geweben poikilothermer Tiere sprechen neue Befunde von ELIAS⁵.

Trotz der weitgehenden Analogie in den Befunden von Tumor- und Kaltblütorgewebe bleibt es aber fraglich, ob die Entsprechung für Tumorgewebe überhaupt gilt oder auf unseren Experimentaltumor, der eine für Tumorgewebe ungewöhnlich hohe Atmung zeigte, beschränkt werden muss.

¹ A. LOCKER, vorangehende Mitteilung No. 11.

² P. SIEKEVITZ und V. R. POTTER, *Cancer Res.* 13, 513 (1953). – M. WOODS, *J. Nat. Cancer Inst.* 17, 615 (1956).

³ A. LOCKER *et al.*, *Z. exp. Med.* 117, 519 (1951).

⁴ F. H. JOHNSON, H. EYRING und M. J. POLISSAR, *The Kinetic Basis of Molecular Biology* (New-York-London 1954).

⁵ H. ELIAS, *Acta Hepatol.* 5, 1 (1957).

Die Tumoren wurden von Herrn Doz. E. DEUTSCH im hiesigen Laboratorium mit der ihm von weiland Prof. A. MÜLLER übergebenen Substanz erzeugt und zur weiteren Stoffwechseluntersuchung uns freundlicherweise zur Verfügung gestellt.

A. LOCKER und R. HOFER

I. Medizinische Klinik der Universität Wien, 9. Januar 1958.

Summary

(1) The oxygen uptake of an experimental sarcoma of the rat was measured under the influence of 4,6-Dinitro-o-cresol in a range of temperature from 22.5 to 47.5°C.

(2) Dinitrocresol inhibited the respiration of the tumor at low temperatures and stimulated it with rising temperatures, similar to its action on tissues of poikilothermic animals.

(3) An explanation is given in terms of the theory of JOHNSON and our own preceding results.

Die Wirkung der Temperatur auf das Verhalten der Gewebsatmung poikilothermer und homoiothermer Tiere unter dem Einfluss von 4,6-Dinitro-o-kresol

Obwohl seit langem bekannt ist, dass die Gewebsatmung poikilothermer Tiere niedriger ist als die homoiothermer, liegt bis heute keine Untersuchung über die Ursache dieser Unterschiede vor. In dieser Mitteilung wird daher eine Untersuchung über die Gewebsatmung der Leber von Mäusen und Kröten im Temperaturbereich von 17,5 bis 42,5°C vorgelegt, wobei der Einfluss von 4,6-Dinitro-o-kresol (DNOC) im Konzentrationsbereich 10^{-4} bis 10^{-6} M studiert wurde.

Die Gewebsatmungsmessung erfolgte nach dem indirekten Verfahren von WARBURG mit Krebs-Bicarbonat-Ringer als Medium in der für Warm- und Kaltblüter erforderlichen Isotonie. Versuchsdauer 3 h. Weitere methodische Einzelheiten wie in unseren bisherigen Untersuchungen¹.

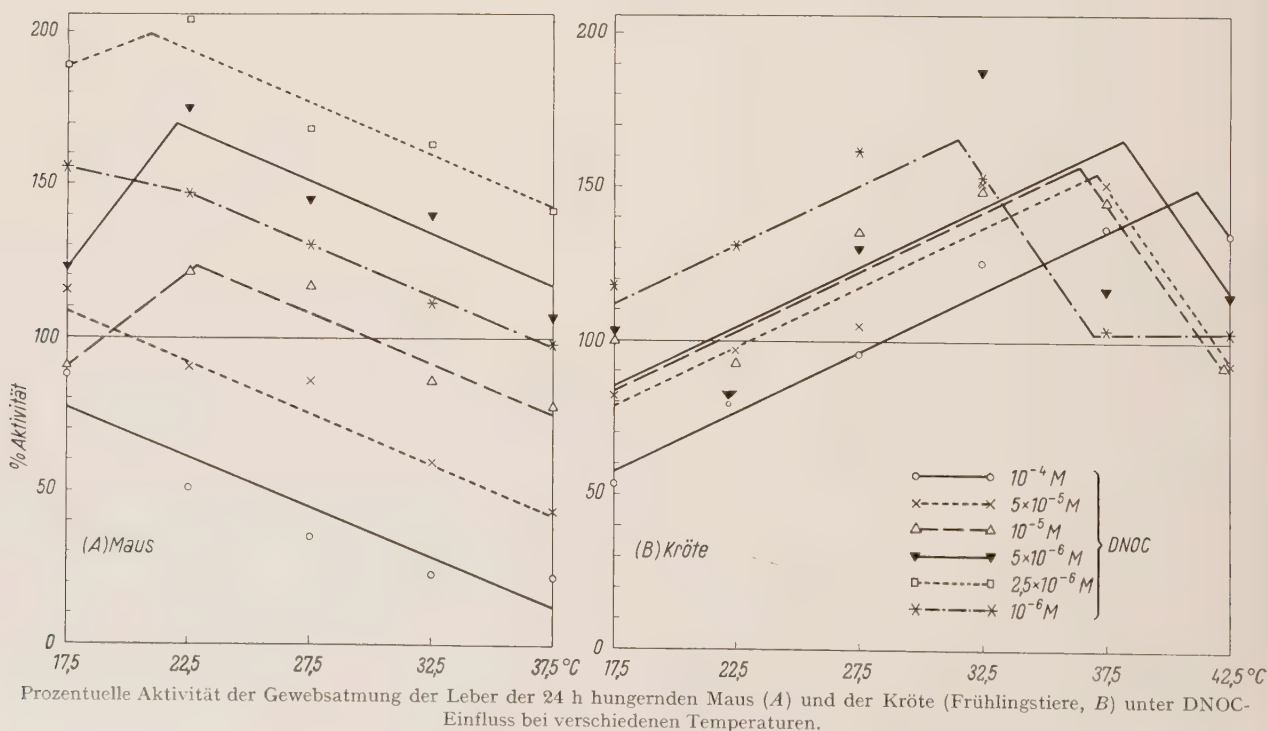
Die Leberatmung zeigt die bekannte Temperaturbeziehung, nämlich allmählichen Anstieg mit einem absoluten Maximum bei 37,5° (Q_{O_2} der Mausleber bei dieser Temperatur: 11,79, der Krötenleber: 1,91). DNOC übt unterschiedliche Wirkungen an den beiden Geweben aus, die gut bei Umformung der Q_{O_2} -Werte in relative Aktivität erfasst werden (Abbildung). Hierbei zeigt sich, dass mit zunehmender Temperatur die Atmung der Mausleber unter DNOC eine zunehmende Verminderung der prozentuellen Aktivität erfährt. Das Umgekehrte ist an der Leber der Kröte der Fall, deren Atmung unter DNOC bei tiefen Temperaturen gehemmt erscheint, aber mit Steigerung der Temperatur eine Förderung erfährt.

Die Aktivierungsenergie (μ) nach ARRHENIUS, berechnet aus der Gewebsatmung im Intervall von 17,5 bis 37,5°C, wird an der Mausleber durch höhere Konzentrationen (10^{-4} M) von DNOC gehemmt ($16900 \rightarrow 3600$ cal), dagegen an der Krötenleber gesteigert ($9300 \rightarrow 17700$ cal).

Diese Ergebnisse können im Lichte der Theorie von JOHNSON² erklärt werden, welche die Zunahme der Aktivität biologischer Systeme mit der Temperatur bzw. ihre Abnahme über dem absoluten Maximum als Folge von Verschiebungen an reversiblen Gleichgewichten ansieht, die an den Enzymen der Zelle zwischen aktiver und inaktiver Form bestehen sollen. Diese Gleichgewichte werden durch chemische Wirkstoffe beeinflusst, wobei 3 typische Hemmformen entstehen, mit denen sich unser Ergebnis konfrontieren lässt. Das Verhalten der Gewebsatmung der Krötenleber nach DNOC (Zunahme der prozentuellen Aktivität mit Erhöhung der Temperatur) ent-

¹ A. LOCKER et al. Z. exper. Med. 117, 519 (1951).

² F. H. JOHNSON, H. EYRING und M. J. POLISSAR, *The Kinetic Basis of Molecular Biology* (New-York-London 1954).



spricht dem Hemmtyp I, das heisst einer Bindung des Inhibitors an natives *und* denaturiertes Enzym, während die %-Aktivitäts-Temperatur-Relation der Mauseleber mit dem Typ II oder III, die kinetisch voneinander nicht unterscheidbar sind, im Einklang steht, das heisst mit einer Bindung des Inhibitors an nur *eine* (entweder native oder denaturierte) Enzymform. Das unterschiedliche Verhalten unter ein und demselben Agens an der Leber von Maus und Kröte ist nur unter der Annahme zu verstehen, dass bei diesen Tieren die Enzymgleichgewichte nicht in derselben Weise existieren. Es muss angenommen werden, dass DNOC auf beide Eiweissformen wirkt. Der Hemmtyp I kommt im Gewebe des poikilothermen Tieres effektiv zur Geltung, weil sich im reversiblen Enzymgleichgewicht beide Seiten konzentrationsmässig entsprechen. Der Typ II (oder III) tritt im Gewebe des homoiothermen Tieres dadurch in Erscheinung, dass hier ein zu Gunsten *einer* Phase extrem verschobenes Gleichgewicht vorliegt, und zwar, wie aus der absolut höheren Aktivität geschlossen werden muss, zu Gunsten der *aktiven* Phase. Auch die Veränderungen der Aktivierungsenergie deuten an, dass an der Mauseleber ursprünglich aktives Enzym vorliegt, welches durch hohe Konzentration an DNOC nur denaturiert werden kann, während die Zunahme der Aktivierungsenergie unter der gleichen DNOC-Konzentration an der Krötenleber eine Verschiebung auf die aktive Seite des Gleichgewichts ausdrückt. Hierin offenbart sich ein Verhalten, wie es für die Beeinflussung metabolisierender Steady-State-Systeme bekannt ist³, was zugleich eine Erweiterung der Johnson-

³ J. Z. HEARON, *Physiol. Rev.* 32, 499 (1952). – L. V. BERTALANFY, *Biophysik des Fließgleichgewichtes* (Braunschweig 1953). – A. LOCKER, Vortrag Österr. Biochem. Gesellschaft, 20. Mai 1957 (im Erscheinen).

schen Theorie, die in ihrer bisherigen Form nur auf dem Massenwirkungsgesetz beruht, bedeuten würde. Auch die relativen Maxima, die als Übergang von Hemmtyp I zu II bzw. III aufgefasst werden können (Abb.: etwa bei 22,5° an der Mauseleber und zwischen 32,5 und 37,5° an der Krötenleber) erfordern zu ihrer Erklärung eine Steady-State-Kinetik. Diese kann in Verbindung mit jenen Gleichgewichten gedacht werden, die MANDELSTAM⁴ in der Kinetik adaptiver Enzyme annimmt.

Herrn Dr. K. H. SPITZY danke ich für die Bereitstellung von Mitteln aus dem Fonds des Antibiotica-Forschungslaboratoriums an der hiesigen Klinik zur Durchführung der vorliegenden Untersuchung.

A. LOCKER

I. Medizinische Klinik der Universität Wien, 9. Januar 1958.

Summary

- (1) The liver respiration *in vitro* of mice and toads was studied under the influence of 4,6-Dinitro-o-cresol at temperatures varying from 17,5 to 42,5°C.
- (2) The percentage activity of the oxygen uptake of the mouse liver decreased progressively under dinitrocresol with rising temperatures, whereas the respiration of the toad liver showed inhibition at low temperatures and activation at higher temperature.
- (3) The results were interpreted following the theory of inhibitory types of JOHNSON.

⁴ J. MANDELSTAM, *Biochem. J.* 51, 674 (1952).

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Mechanik der Erde

Elemente und Studien zur tektonischen Erdgeschichte

Von Dr. RICHARD A. SONDER

Mit 91 Abbildungen und 18 Tabellen im Text auf 11 Beilagen, 291 Seiten

(E. Schweizerbart'sche Verlagsbuchhandlung, Stuttgart 1956)

(Nägele u. Obermiller)
(DM 42.—)

Das Werk stellt sich die gewaltige Aufgabe, das Gesetzmässige des geomechanischen Geschehens, bei möglichst erschöpfender Ausnützung aller einschlägigen Befunde und Zahlenwerte, aufzuzeigen und unter plausiblen Grundannahmen aus den allgemeinen Gesetzen der mechanischen Beanspruchung und Verformung zu erklären. Voraussetzung für dieses Unterfangen ist ein ungewöhnlicher Überblick über Kenntnisstand und Gedankengut der verschiedensten mit unserem Planeten sich befassenden Disziplinen und ein Vertrautsein mit ihren jeweiligen Methoden. Paläogeographische, paläontologisch-entwicklungsgeschichtliche, stratigraphische, ozeanographische, klimatologische, vulkanologische, petrographisch-mineralogisch-geochemische Einzelheiten müssen in vielfältiger gegenseitiger Verknüpfung geotekto-

nisch bzw. geomechanisch interpretiert werden. Bei der Fülle der Anforderungen ist es nicht verwunderlich, dass die Aufgabe im hier vorliegenden Umfang bisher noch nicht anzugehen gewagt wurde, so dass der Verfasser allenthalben auf sich selbst gestellt in Neuland vorstösst. Man könnte sich eine Lösung derart denken, dass unter strengster Prüfung der verwendeten Materialkonstanten und sonstigen Zahlenwerte und der erforderlichen Extrapolationen versucht wird, bei vorsichtigem Abwägen *sine ira et studio* das Sichere, das Wahrscheinliche und das Mögliche voneinander zu trennen. Der Verfasser wählt – mit Recht wohl seinem eigenen Temperament folgend – den anderen Weg: er stellt sein reiches Wissen in den Dienst einer einzigen, gewiss diskussionswürdigen Hypothese (der einer langsamen stetigen Erdkontraktion) und vermag so ein durch seine Geschlossenheit imponierendes Gebäude zu errichten. Neben anderen schon angedeuteten Tugenden kommt ihm dabei eine wahre Souveränität in der begrifflichen Erfassung und Aufgliederung weitläufigerer Tatbestände zugute, sowie der Mut zu verantwortlichem Abschätzen, wo es nötig wird. Eine unvermeidliche Einseitigkeit der Darstellung wird mehr als ausgeglichen durch die überall spürbare überzeugende Wärme und eine Sicherheit in der Handhabung der Argumente, wie sie doch wohl nur aus der jahrelangen Absicherung des gewählten eigenen Standpunktes erwachsen kann.

Die Verarbeitung eines umfangreichen Tatsachenmaterials zwingt zu ausgiebigem Gebrauch graphischer Darstellungen; an Diagrammen, Profilen, Karten, Tabellen und Listen wurde nicht gespart. Viele instruktive Zusammenstellungen stammen dabei vom Verfasser selbst. Auch sonst lässt die Ausstattung des Buches nichts zu wünschen übrig.

E. BAIER

Erwachende Wissenschaft

Von B. L. VAN DER WAERDEN

484 Seiten mit 200 Figuren

Sammlung «Wissenschaft und Kultur», Band 8

Birkhäuser Verlag Basel und Stuttgart, 1956

Ganzleinen Fr. 37.50

In VAN DER WAERDENS «Erwachende Wissenschaft» haben wir eine ausgezeichnete Darstellung der Geschichte der Mathematik des Altertums vor uns, deren Probleme, gegründet auf den neuesten Forschungsergebnissen, in einer übersichtlichen und verständlichen Form verarbeitet und zueinander in Beziehung gebracht werden. Das Werk soll, wie der Verfasser in der Einführung bemerkt, sowohl wissenschaftlich exakt, als auch leicht fasslich und dem Absolventen einer Mittelschule zugänglich sein; man wird zu einer Mitarbeit aufgefordert durch den Satz: «Glaubt mir nichts, prüft alles nach!» In der Tat stösst der interessierte Leser auch auf mancherlei Stellen, die ihn zum selbständigen Nachdenken anregen mögen.

Das Werk zerfällt in acht Kapitel, denen eine Einleitung vorausgeschickt ist, in der der Verfasser auf die Notwendigkeit des Studiums der Geschichte der Mathematik hinweist. Diese Notwendigkeit wird an Hand einiger, auch dem Laien einleuchtender Beispiele aus der abendländischen Kultur, der Technik und dem täglichen Leben vor Augen geführt (wobei nur NEWTON allein als derjenige genannt wird, auf dem die Differential- und Integralrechnung beruht).

Das Studium der einzelnen mathematik-historischen Probleme wird besonders erleichtert durch eine glückliche Wahl von Abbildungen und Übersetzungen von Originaltexten. In dieser Hinsicht steht das Werk in der deutschen Fachliteratur einzig da.

In Kapitel I wird die ägyptische Mathematik behandelt, mit einer Fülle von Zitaten und Quellenangaben, wobei die uns überlieferten griechischen Quellen einer scharfen Kritik unterzogen werden. Es wird die Ansicht vertreten, dass die ägyptische Mathematik, entgegen vieler früherer Annahmen, nicht den Ausgangspunkt für die griechische gebildet hat, der Inhalt der uns überlieferten ägyptischen Texte wird (S. 57) als «Rechenvorschriften ohne jegliche Motivierung» bezeichnet und dieses durch den Satz: «Aber Rechnen ist noch keine Mathematik» unterstrichen, eine Gegenüberstellung, die für mathematik-historische Untersuchungen zunächst bedenklich erscheint. Doch im folgenden Kapitel II bringt ja der Verfasser die sehr wünschenswerten Ausführungen über «Zahlensysteme, Ziffern und Rechenkunst», was als Problemgeschichte für den Zeitraum von den Babyloniern bis zum Mittelalter verfolgt wird. Es wird darin das Vorhandensein einer geeigneten Zahlenschreibweise und Rechentechnik immerhin als unumgängliche Bedingung für die Entwicklung der Mathematik gekennzeichnet. In Kapitel III wird die babylonische Mathematik anhand der neuesten Forschungsergebnisse ausführlich behandelt und als Grundlage für die griechische Mathematik charakterisiert. Dies betrifft

insbesondere den pythagoreischen Lehrsatz, von dessen Kenntnis uns mehrere Keilschrifttexte berichten. Die uns überlieferten Lösungen der quadratischen Gleichungen der Babylonier sind durch verschiedene Autoren unseres Jahrhunderts auf mannigfaltige Weise interpretiert worden; es stand hier unter anderem die Fragestellung, ob den Babyloniern die Doppeldeutigkeit der Quadratwurzel bekannt gewesen ist, im Vordergrund. Hierzu bringt der Verfasser Übersetzungen und Deutungen mehrerer altbabylonischer Keilschrifttexte.

Der weitaus grösste Teil des Werkes ist der griechischen Mathematik gewidmet, wobei die Frage nach dem Einfluss des alten Orients besondere Beachtung findet. Auch hier wird mancher Leser wiederholt auf solche Ausführungen stossen, die nicht mit dem übereinstimmen, was er sonst darüber erfahren haben mag. So heisst es unter anderem auf Seite 168, dass wir über die Geometrie des PYTHAGORAS, «wenn man es recht betrachtet, überhaupt nichts» wissen. Freilich, die direkten Quellen sind sehr selten, doch wird durch allgemeine historische Überlegungen hierzu eine auf PYTHAGORAS führende Entwicklung wahrscheinlich gemacht, so dass die Skepsis nicht übertrieben werden sollte.

Kapitel IV beginnt mit den frühesten uns bekannten Überlieferungen der griechischen Mathematik, dem Jahrhundert des THALES und PYTHAGORAS. In Kapitel V behandelt der Verfasser das «Goldene Zeitalter», das 5. Jahrhundert, wo die mathematischen Erkenntnisse einer Systematisierung unterzogen wurden und die Zeit der grossen Schulen begann; es schliesst mit dem Problem des Irrationalen. Es folgt in Kapitel VI die Zeit PLATONS. Hier sehen wir besonders deutlich die Wechselwirkung der damaligen geistigen Strömungen auf die Mathematik, insbesondere von der Philosophie her. Den Schluss dieses Kapitels bildet EUKLID von Alexandria, nachdem schon in den vorausgehenden Kapiteln sehr eingehende Betrachtungen darüber angestellt werden, welchen Autoren die einzelnen Bücher seiner «Elemente» zuzuschreiben sind. Hier trifft man wieder einige Aussagen, die auch wahrscheinlich mehr dazu gedacht sind, den Leser zu eigenen Überlegungen anzuregen, als ein abgeschlossenes Urteil darzustellen. So heisst es zum Beispiel auf Seite 322 über EUKLID: «Er ist der grösste Schulmeister, den die Geschichte der Mathematik kennt.» Und weiter auf Seite 323: «EUKLEIDES ist bestimmt kein grosser Mathematiker.» In Kapitel VII sehen wir die alexandrinische Zeit, zu der der grösste Mathematiker des Altertums, ARCHIMEDES, gehört, welchem eine besonders ausführliche Darstellung gewidmet ist. Das Werk schliesst mit den Untersuchungen (Kapitel VIII) der Ursachen der griechischen Mathematik.

Es ist dankenswert, dass VAN DER WAERDEN das ungeheure Material, das die Forschung in den letzten Jahrzehnten auf dem Gebiete der Geschichte der Mathematik des Altertums zutage gefördert hat, in einer verständlichen Weise zu verarbeiten vermochte und es unternahm, diesen Stoff mit all seinen einzelnen Ergebnissen einer so interessanten und tiefgreifenden Gegenüberstellung zu unterziehen. Besonders sei hervorgehoben, dass der Verfasser darauf bedacht war, die Mathematik in ihrer Verknüpfung mit den anderen Bestandteilen des geistigen Lebens zu behandeln, ist ja die Mathematik doch eine der vielen Erscheinungen der allgemeinen Kulturgeschichte. So verdient das Werk weiteste Verbreitung; es ist sowohl dem interessierten Laien als auch dem Studierenden sowie dem Dozenten unentbehrlich und verlockt dazu, die Geheimnisse einer erwachenden Wissenschaft mit den zeitgemässen Mitteln zu erforschen.

N. STULOFF

Informations - Informationen - Informazioni - Notes

STUDIORUM PROGRESSUS

The Relation Between Activity and Blood Flow in the Thyroid Gland¹

By U. SÖDERBERG*

Since the famous remark by KING² that the nourishment of the thyroid gland 'does not seem to be the main intention of its vascular supply', a great number of experiments have been performed measuring blood flow as an index of thyroid activity. Thus, OSSOKIN, WATTS, and GUNNING³ introduced the measurement of venous outflow from the gland, a method which has been adapted to the technique described below. The discovery of the iodine metabolism at the beginning of this century was a beautiful confirmation of KING's suggestion as it showed that the intimate contact between thyroid cells and the circulating blood was an effective arrangement for filtering off the small amounts of iodide present in the blood. Synthesis and transportation of a few micrograms of thyroid hormone per day to the systemic circulation would probably have been managed at a much reduced rate of blood flow.

The introduction of radioactive isotopes of iodine in thyroid physiology⁴ concentrated the main interest on direct measurements of the level of iodine in the gland and earlier circulatory studies seem to have been forgotten. However, it is still of considerable importance to collect information about the relation between blood flow and uptake of iodine for a correct understanding of the regulation of thyroid functions. Although the iodide clearance of the thyroid has been determined indirectly from the thyroid increment of radioactivity and blood concentration of radioiodide by e.g. BROWN-GRANT *et al.* and BROWN-GRANT and GIBSON⁵ and thyroid blood flow has been calculated from the thyroid A-V difference of radioiodine and indirect clearance determinations (POCHIN⁶) no sincere attempt at direct measurement of thyroid blood flow and A-V difference simultaneously seems to have been reported in the literature.

The technique to be described was also found accurate enough for measuring the release of labelled thyroid hormone. As it could be demonstrated that rate of secretion of hormone did not correlate with rate of blood flow, isolated measurements of the content of hormone in arterial and thyroid venous blood that have been previously reported give very little information of thyroid activity. The present experiments have shown that the

combining of the measurement of venous outflow from the gland with assay of inorganic radioiodide and protein bound radioiodine in the systemic arterial blood and in the venous blood from the thyroid gland made it possible to record much more rapid changes than those seen in previously reported experiments in which changes in the total content of radioiodine in the thyroid or in the circulating blood are followed.

As the indices used here for thyroid functions were particularly sensitive to short term reactions, special attention was directed towards the somewhat obscure effects of nervous activity and blood catechols on the thyroid which have been described by several authors⁷.

Methods. Experiments were performed on 18 cats and 66 rabbits under light to moderate anaesthesia ('Nembutal', 'Pentothal', chloralose or urethane). A few cats were *encéphale isolé* or decerebrated. The femoral vein was cannulated for intravenous infusions, one femoral artery for blood pressure recordings with an electromanometer and the other femoral artery for taking blood samples. In some cases the electroencephalogram and other vegetative variables than those described below were also registered.

The animals were completely heparinized. A thin polyethylene cannula was introduced into the largest thyroid vein and all the other veins from the gland were then ligated. The thyroid tissue was dissected as free as possible from its surroundings and covered with mineral oil or octon. Each drop of blood from the free end of the cannula gave a signal to an electric ordinate recorder registering blood flow (see Legend to Fig. 1) and fell on a slowly running strip of filter paper. These strips were then dried and afterwards analyzed for radioiodine by a scintillation counter. Drops of arterial blood were examined in a similar way. Known volumes of venous and arterial blood were also repeatedly tested for radioactivity to permit accurate determination of the relation between the two series of drops. Thus, blood flow and arterio-venous concentration difference could be continuously recorded with a fairly high degree of accuracy and with good temporal resolution, the latter being related to time interval between drops. After corrections for the volume of the recording system had been done, these two factors, blood flow and A-V difference, provided all data necessary for the calculation of the rate of uptake of iodine and the rate of release of hormonal radioiodine. No distinction has been made here between the various thyroid hormones⁸, and, for simplicity, it is assumed that there is a uniform labelling of the hormones in most of the cases.

After a short period of recording the thyroid blood flow, one or several tracers of radioiodine was injected intravenously. For determinations of the rate thyroid hormone secretion, the tracer was administered 1 to 3 days before the acute experiment, so that the hormone was labelled with radioiodine. Since the blood drop technique permitted repeated measurements of the radioactivity of all the blood samples, several isotopes of iodine with different half-lives could be used simultaneously. Consequently, both the rate of uptake of iodine and release of hormone could be measured in the same ex-

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¹ The research reported in this paper has been sponsored by the Office of Scientific Research of the Air Research and Development Command, United States Air Force, through its European Office, under Contract AF 61 (514) - 1083.

² T. W. KING, *Guy's Hosp. Rep.* 1, 429 (1836).

³ N. OSSOKIN, *Z. Biol.* 63, 443 (1914). - C. F. WATTS, *Amer. J. Physiol.* 38, 356 (1915). - R. E. L. GUNNING, *Amer. J. Physiol.* 44, 215 (1917).

⁴ S. HERTZ, A. ROBERTS, and R. D. EVANS, *Proc. Soc. exp. Biol. Med.* 38, 510 (1938). - J. G. HAMILTON and M. H. SOLEY, *Amer. J. Physiol.* 127, 557 (1939). - C. P. LEBLOND, P. SUE, and A. CHAMORRO, *C. R. Soc. Biol.* 133, 540 (1940).

⁵ K. BROWN-GRANT, C. VON EULER, G. W. HARRIS, and S. REICHLIN, *J. Physiol.* 126, 1 (1954). - K. BROWN-GRANT and J. G. GIBSON, *J. Physiol.* 127, 328 (1955).

⁶ E. E. POCHIN, *Lancet* 259, 41, 84 (1950).

⁷ See e.g. W. B. CANNON and M. CATTELL, *Amer. J. Physiol.* 41, 39, 58, 74 (1916) - H. B. FRIEDGOOD and W. B. CANNON, *Endocrinology* 26, 142 (1940).

⁸ J. GROSS and R. PITT-RIVERS, *Biochem. J.* 53, 652 (1953). - J. ROCHE, S. LISSITZKY and R. MICHEL, *C. R. Acad. Sci., Paris* 232, 2047 (1952). - A. TAUROG, J. D. WHEAT and I. L. CHAIKOFF, *Trans. Amer. Goiter Ass.* 228 (1955).

periment and studied in relation to each other and to blood flow. Figure 1 illustrates the results of such an experiment. It shows the influence of 4 μ g adrenaline on the secretion of thyroid hormone labelled with I^{131} (half-life 8 days) and uptake of I^{132} (half-life 2.33 h)⁹. The multi-tracer technique was also used for differentiating diffusion of iodine from 'active' uptake.

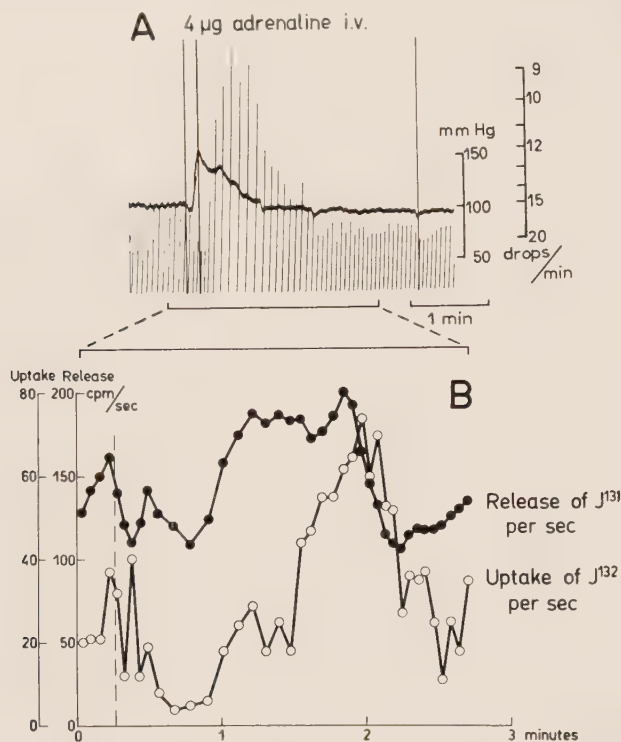


Fig. 1.—Male rabbit, 2.5 kg. Nembutal and chloralose. *A* Blood pressure from the femoral artery (heavy horizontal line) and blood flow from a cannula in a large thyroid vein (steeply rising lines). Each drop of blood from the free end of the thyroid cannula makes a signal which rapidly carries the spot of the mirror galvanometer to the base of the record from which it returns at slower speed so that a steeply rising line is recorded. The length of these lines is directly proportional to time-interval between drops. By joining the upper ends of these lines one obtains a curve inversely proportional to thyroid blood flow (cf. calibration on the right). *B* Rate of uptake of I^{132} (open circles) and rate of release of hormone labelled with I^{131} (filled circles). Calibration on the left in counts per min per s (cpm/s) i.e. radioactivity taken up or released per unit time. The time relation between *A* and *B* is illustrated by horizontal bars between the figures. Vertical broken line in *B* at the onset of adrenaline injection. Adrenaline injected intravenously (4 μ g) constricts thyroid vessels with short latency, increases rate of release of hormonal I^{131} with short latency and increases rate of release of hormonal I^{131} with a latency somewhat less than 1 min. Rate of uptake of I^{132} is reduced initially but is then increased above the initial level. Secretion of thyroid hormone was induced by administration of large doses of exogenous thyrotropic hormone ('Actyron').

Results. In a large series of experiments where circulatory changes in the thyroid were elicited by central or peripheral nervous stimulations or by administration of hormones or drugs, it was found that the rate of uptake of iodine was largely proportional to the blood flow through the gland. Thus, the arterio-venous concentration difference of inorganic radioiodide was often found to be roughly constant during considerable length of time also when there were moderate changes in blood flow.

During severe vasoconstrictions the A-V difference fell and it increased during large dilatations. The uptake was somewhat augmented when the animals were breathing pure oxygen and decreased during inhalation of gas mixtures rich in carbon dioxide or poor in oxygen. The rate of uptake was, however, less reduced after large doses of antidiuretic hormone than one would have expected from the vasoconstriction. Another exception from the simple relationship was observed in animals pretreated with heavy doses of thyrotropic hormones (TSH)¹⁰ after administration of adrenaline or after sympathetic stimulation. Under these conditions, there was often a period of increased rate of uptake during the recovery from the stimulation (Fig. 1). This effect was obtained with a latency as short as a few minutes after the injection of the thyrotropic hormone and is, in fact, the earliest effect of injected TSH so far known. It does not seem to be closely related to the well-known long-latency effect of TSH on iodine accumulation. Corresponding to the known inhibition of the release of TSH from the anterior pituitary, following 1 to 3 days after the administration of large amounts of thyroxine (EULER and HOLMGREN¹¹), there was a strong reduction of the rate of uptake of iodine (down to zero). However, at the same time the thyroid blood flow was largely unaffected.

In order to release the radioiodide that was trapped by the thyroid gland but not yet utilized, thiocyanate or a heavy dose of NaI^{127} was administered. It was thereby observed that the turnover rate of iodine within the gland was generally reduced during the acute experiments, probably by influence of the anesthetics.

The rate of secretion of thyroid hormone, in contrast to the rate of uptake of inorganic iodide, was found to follow what was believed to be the titre of endogenous TSH or administered exogenous thyrotropic hormone regardless of the blood circulation through the gland. Consequently, the thyroid V-A concentration difference of labelled thyroid hormone increased considerably during periods of vasoconstriction and decreased during vasodilatation. Secretion of hormone and vasomotor activity were generally not parallel. The release of thyroid hormone, maintained by the TSH, could be modified by various means. Thus, injection of adrenaline, noradrenaline or acetylcholine, electrical stimulation of the cervical sympathetic trunk or the peripheral end of the cut superior laryngeal nerve were as a rule followed by a transient increase of the release of labelled hormone per unit time, as is also seen in Figure 1 (filled circles). A brief nerve stimulation generally produced a much more long-lasting effect on the rate of release of thyroid hormone than a single injection of adrenaline, noradrenaline or acetylcholine regardless of the vasomotor response to the same stimulus. These observations permit one to suggest the existence of nerve fibres that can markedly change the rate of output of thyroid hormone in response to a given input of TSH. This suggestion was verified by experiments where the veins from either of the two isolated halves of the gland were cannulated. If one half of the gland was acutely denervated and the peripheral end of the cut superior laryngeal nerve stimulated there was an increase of the rate of release of hormone into the vein from that half of the thyroid gland. The other half, with intact innervation, could be stimulated to increased secretion by nervous reflexes or by drugs acting via the central nervous system as in Figure 2 were the release is elicited by 2-brom-lysergic acid diethylamide, 'BOL-148'

⁹ I^{132} was obtained by the courtesy of Dr. L.-G. LARSSON and Dr. J. EINHORN, Radiumhemmet, Karolinska Sjukhuset, Stockholm (Sweden).

¹⁰ TSH, 'Actyron', was kindly supplied by A. B. Ferring, Malmö (Sweden).

¹¹ C. von EULER and B. HOLMGREN, *J. Physiol.* 131, 125 (1956).

(CERLETTI and ROTHLIN¹²). Adrenaline injected intravenously had inconstant effects in the animal of Figure 2. In another rabbit a heavy spontaneous 'arousal reaction' was followed by a more pronounced increase in the thyroid hormone release from the intact half of the gland than from the acutely denervated one. This indicates that nervous influence on the thyroid may be even more important than the effect of an increase of blood catechols during 'arousal'. Such experiments can, however, only be performed in animals in which the vascular anatomy is extremely favourable.

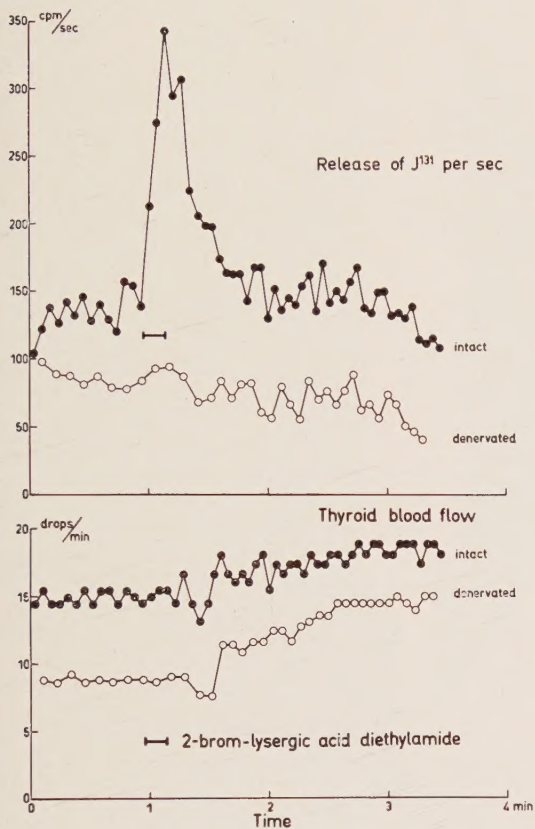


Fig. 2.—Male rabbit, 3.0 kg, Nembutal. Hormone secretion induced by exogenous TSH ('Actyron'). Upper part: Rate of release of hormonal I¹³¹ as in Figure 1B from intact and acutely denervated halves of thyroid. Lower part: Rate of venous outflow from the same halves. Brom-lysergic acid diethyl amide ('BOL-148') increases rate of release by more than a 100% in the intact half of the gland. Blood flow increases slightly in both halves. Blood pressure (not reproduced in the figure) showed slight transient fall.

It has thus been found that rate of uptake of iodine and rate of release of thyroid hormone may be widely independent of each other in the acute experiment. More experimentation has, however, to be done to permit an analysis of the long-time dependence that evidently exists.

The results show that changes in thyroid activities may be more rapid than was expected and that thyroid activity may be a useful index of vegetative changes both in physiological and pharmacological experiments even in animals subjected to small operations under anesthesia.

¹² A. CERLETTI and E. ROTHLIN, *Nature* 176, 785 (1955). — E. ROTHLIN, *J. Pharm. Pharmacol.* 9, 569 (1957). — The author is indebted to Sandoz AG., Basel (Switzerland) for 'BOL-148'.

Nervous and hormonal stimuli have been shown to have marked effects on thyroid hormone secretion. However, these effects are due to changes in the responsiveness to TSH and cannot be demonstrated in the absence of the pituitary hormone. The uptake of TSH by the thyroid gland may also be related to thyroid blood flow governed both by nerves and hormones.

A detailed description of this work will be presented in the near future¹³.

Note added during correction of proof: Since this paper was sent to the Editor, a description of measurement of thyroid blood flow from thyroid I¹³¹ clearance and A-V difference in the rabbit has been presented in *Amer. J. Physiol.* 192, 268 (1958) by E. F. MONKUS and E. P. REINEKE. Their work beautifully supports the view that thyroid blood flow and the effect of TSH do not always go hand in hand. In a group of rabbits with mean acinar cell height of 4.7 μ the A-V difference was 25%, in another group with mean cell height of 7.9 μ the A-V difference averaged 57%.

Zusammenfassung

An 18 Katzen und 66 Kaninchen wurde die Beziehung zwischen Schilddrüsendurchblutung, Aufnahme von Jod in die Schilddrüse und Abgabe von Schilddrüsenhormon untersucht. Das anorganische und das an Eiweiss gebundene Jod wurde im arteriellen und im venösen Blut der Schilddrüse mit Hilfe radioaktiver Isotopen bestimmt. Die Durchblutung wurde als Frequenz der von dem freien Ende einer in der Schilddrüsenvene befindlichen Kanüle fallenden Tropfen registriert. Es zeigte sich, dass die Geschwindigkeit der Jodaufnahme im wesentlichen proportional der Durchblutung ist, während die Geschwindigkeit der Abgabe von hormonalem Jod durch das thyreotrope Hormon der Hypophyse (TSH) bestimmt wird. Nervöse und hormonale Reize führen auf dem Weg über Durchblutungsänderungen innerhalb kurzer Zeit zu einer Veränderung der Aufnahmegeschwindigkeit und beeinflussen ausserdem die Hormonabgabe durch Änderung der Empfindlichkeit der Schilddrüse gegenüber dem TSH. Die Aufnahme von TSH in die Schilddrüse hängt wahrscheinlich von der Durchblutung ab. Bromlyserginsäurediäthylamid, das an der intakten Schilddrüse die Abgabe von hormonalem radioaktivem Jod erhöht, ist an einer akut denervierten Schilddrüsenhälfte ohne Wirkung; daraus kann geschlossen werden, dass die Schilddrüsennerven eine fördernde Wirkung auf die Hormonfreisetzung haben.

¹³ U. SÖDERBERG, *Acta physiol. Scand.* 42, Suppl. 147, (1958).

CONSTRUCTIONS

An Experiment in the Design of a Scientific Conference

The purpose of this note is to report on a conference design that differed somewhat from the traditional plan of scientific meetings, and on its possible value for similar scientific conferences.

As science progresses, as the number of scientists increases and as scientists become more specialized, the problem of communication among scholars becomes more and more pronounced. The large scientific conventions and the international congresses that several decades ago

provided an adequate form for technical discussion are no longer performing this function because of the tremendous increase in the number of participants and of the ensuing limitations of time. For the last twenty years or so symposia, i.e., scientific meetings with limited attendance and with only invited speakers, have been used more frequently because of the advantages offered by bringing together a relatively small number of specialized scientists. The climate of such gatherings is more conducive to a high level of technical discussions than the larger and more heterogeneous scientific congresses. When, however, the symposium has a relatively large number of participants and their primary interests are diverse, a problem of communication arises. One such problem was faced by the organizers of the international symposium on 'Perspectives in Marine Biology', held at the Scripps Institution of Oceanography of the University of California at La Jolla, California.

The primary purpose of this conference was that of identifying those areas of investigation that could be most profitably attacked at the experimental level in the field of marine biology. For this aim it appeared necessary to invite a number of biologists representing a wide range of specialized interests, such as marine and non-marine ecologists, physiologists, microbiologists, biochemists, etiologists, geneticists and evolutionists. The actual participation was as follows: 47 invited participants, 131 observers; 14 nations were represented. In developing the conference design, the planners considered the following problems which were inherent in the nature of the symposium: (1) the difficulty of communication between specialists in the different scientific fields represented; (2) language problems because of the different nationalities represented; (3) the tendency of some scientists to focus on single areas of research rather than on broad problems of the total field; (4) the reluctance of some scientists to explore unfamiliar grounds in the presence of their colleagues; and (5) the tendency of specialists in a particular field of science to seek out and converse with colleagues from the same field rather than from other disciplines.

In one respect this conference was like many other symposia: scientists were invited to prepare papers in advance and read them at the meeting; each formal presentation was followed by a discussion of the issues raised by the speaker, to which all the attendants, invited and observers, were free to participate. However, in order to provide a setting in which interdisciplinary thinking could better take place and where new areas of research might be found, this particular symposium included another element: the 'Idea Group'. Six such groups, consisting of eight or nine invited participants, plus a discussion leader and a recorder, met periodically throughout the conference. Each 'Idea Group' included scientists from the different fields represented at the conference. These heterogeneous groups met every noon for lunch and several other times during the symposium. Their stated purpose was that of dealing with the broad question around which the entire symposium was built: What are now the most promising areas of experimental research in the field of marine biology? The 'Idea Groups' were instructed to use the formal presentations as background and as a springboard for discussing this general problem. The discussion leaders and recorders of each 'Idea Group' were members of the staff of the host Institution. A week prior to the conference, these leaders and recorders met to be oriented to the conference design, the purpose of the 'Idea Groups', methods to get them under way, and the roles the leaders and the recorders were expected to play.

The recorders prepared a written summary of the subjects discussed during each working session of the groups; the highlights of these discussions were also reported to all the members of the symposium at the final session by a panel including one person from each 'Idea Group'.

At the end of the conference each participant was asked to fill out an evaluation form in which questions were asked about the value of this conference design.

In spite of some doubts expressed at the beginning of the conference about the value of the 'Idea Group', the participants at the end expressed general satisfaction with this experience and felt that this kind of conference design would be useful in other scientific conferences of this type. The planned heterogeneity of the groups appeared to be useful and appreciated in that it provided a fine opportunity for participants in the conference to become better acquainted with colleagues from other disciplines. Probably the term 'Idea Group' was not very fortunate; a more appropriate name might be adopted in future conferences. A thorough orientation of the leadership (leader and recorder) prior to the conference appears essential for the success of this technique. That this design has a definite value in symposia of this kind follows also from the fact that one of the participants used it successfully in the organization of another scientific conference held in France last summer.

A. A. BUZZATI-TRAVERSO*
and W. H. SCHMIDT

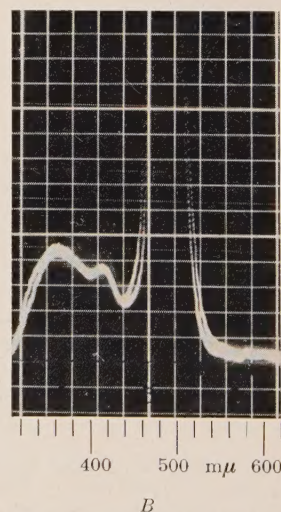
Scripps Institution of Oceanography, La Jolla, California and Department of Conferences and Special Activities, University of California, Los Angeles, May 9, 1957.

* Gegenwärtige Adresse: Istituto di Genetica, Pavia.

Corrigendum

Å. BERTLER, A. CARLSSON, MARGIT LINDQVIST, and T. MAGNUSSON: *On the Catechol Amine Levels in Blood Plasma after Stimulation of the Sympathoadrenal System*. *Exper. 14*, fasc. 5, 184 (1958).

There has been an error in the scale of the Figure B on page 185; corrected scale see below:





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Neuerscheinung

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